69861 U.S. PTO 09/25/97

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PATENT
Atty. Docket No.CRP-070FWCN2
(2054/39)

65705 U.S. PTO 08/937756 09/25/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Rueger, et al.
SERIAL NO.:	-----
FILED:	Herewith
TITLE:	Morphogen-Induced Nerve Regeneration and Repair
GROUP NO.:	1812 (Prior Application)
EXAMINER:	R. Hayes (Prior Application)

CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. 1.10

I hereby certify that the attached document is being deposited with the United States Postal Service, postage prepaid, on September 25, 1997 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service, mailing label number EM354411625US, in an envelope addressed to: ATTN: BOX PATENT APPLICATION, Assistant Commissioner for Patents, Washington, DC 20231.

Brenda L. MacLean
Brenda L. MacLean

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

TRANSMITTAL OF FILING UNDER 37 CFR 1.60(b)

This is a request for filing a Continuation of application under 37 CFR 1.60, of pending prior application serial no. 08/ 260,675 filed on June 16, 1997 of Rueger, et al., for Morphogen-Induced Nerve Regeneration and Repair.

1. **Copy of Prior Application as Filed Which is Attached**

☒ I hereby verify that the attached papers are a true copy of what is shown in my records to be the above identified prior application, including the oath or declaration originally filed (37 CFR 1.60).

The copy of the papers of prior application as filed which are attached are as follows:

147	page(s) of specification
11	page(s) of claims
1	page(s) of abstract
5	sheet(s) of drawing

2. **Amendments**

☒ Cancel in this application original claims 2-81of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

☒ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered **original** claim in the prior application.)

3. **Fee Calculation (37 CFR 1.16)**

CLAIMS AS FILED					
	Number Filed	**Number Extra**		**Rate**	**Basic Fee 37 CFR 1.16(a) $770.00**
Total Claims (37 CFR 1.16 (c))	57	- 20 = 37	X	$ 22.00	$ 814.00
Independent Claims (37 CFR 1.16 (b))	18	- 3 = 15	X	$ 80.00	$ 1200.00
Multiple Dependent Claim(s), If any (37 CFR 1.16(d))	Yes		+	$ 260.00	$ 260.00

☐ Fee for extra claims is not being paid at this time. (37 CFR 1.16(d))

Filing Fee Calculation $ 3,044.00

4. **Small Entity Status**

☒ A verified statement that this filing is by a small entity:

☐ is attached

☒ has been filed in the parent application and such status is still proper and desired (37 CFR 1.28(a))

Filing Fee Calculation (50% of above) $ 1,522.00

5. Drawings

☐ Transfer the drawings from the prior application to this application and, subject to item 17 below, abandon said prior application as of the filing date accorded this application.

☒ Drawings are enclosed

☐ formal
☒ informal

6. Assignment

☒ The prior application is assigned of record to <u>Creative BioMolecules, Inc. at Reel No. 6531 and Frame No. 0897.</u>

☐ an assignment of the invention to _____

☐ is attached.

A separate "ASSIGNMENT COVER LETTER ACCOMPANYING NEW PATENT APPLICATION" is also attached.

NOTE: "If an assignment is submitted with a new application, send two separate letters - one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

7. Fee Payment Being Made At This Time

☒ Enclosed

☒ basic filing fee $ <u>1,522.00</u>

8. Method of Payment of Fees

☒ enclosed is a check in the amount of $ <u>1,522.00</u>

☐ charge Account No. 20-0531 in the amount of $ _____
A duplicate of this request is attached.

9. Authorization To Charge Additional Fees

☒ The Commissioner is hereby authorized to charge any deficiencies in fees to Account No. 20-0531.

☒ Address all future communications to:

Patent Administrator
Testa, Hurwitz & Thibeault, LLP
High Street Tower
125 High Street
Boston, MA 02110

Dated: September 25, 1997
Reg. No. 36,989

Tel. No. (617) 248-7013

Thomas C. Meyers
Attorney for Applicants
Testa, Hurwitz, & Thibeault, LLP
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125 High Street
Boston, Massachusetts 02110

389MM2054/39.413021-1

EXPRESS MAIL MAILING LABEL

No. EM354411625US

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08/937756
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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Rueger, *et al.*
SERIAL NUMBER:	Continuation of 08/260,675
FILING DATE:	Herewith
TITLE:	Morphogen-Induced Nerve Regeneration and Repair
GROUP:	1812 (Prior Application)
EXAMINER:	R. Hayes (Prior Application)

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.10

I hereby certify that this correspondence, together with any documents referred to as being attached hereto is/are being deposited with the United States Postal Service as Express Mail, Mailing Label No. EM354411625US, postage prepaid, in an envelope addressed to: Box Patent Application, Assistant Commissioner for Patents, Washington D.C., 20231 on September 25, 1997.

Brenda L. MacLean
Brenda L. MacLean

PRELIMINARY AMENDMENT

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This amendment is submitted concurrently with the filing of a continuation application for the above referenced patent application. Consideration of the application in view of the amendments made herein respectfully is requested.

In the Claims:

Kindly cancel claim 1 and insert new claims 82-104 as follows:

--82. A method of treating amyotrophic lateral sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

83. A method of treating multiple sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

84. A method of treating a spinal cord injury, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

    (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
    (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
    (c) defined by Generic Sequence 7, SEQ ID NO: 4;
    (d) defined by Generic Sequence 8, SEQ ID NO: 5;
    (e) defined by Generic Sequence 9, SEQ ID NO: 6;
    (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
    (g) defined by OPX, SEQ ID NO: 3,

    wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

85. The method of claim 84, wherein said spinal cord injury results from a mechanical trauma.

86. The method of claim 84, wherein said spinal cord injury results from a tumor.

87. The method of claim 84, wherein said spinal cord injury results from a chemical trauma.

88. A method of restoring motor function in a mammal afflicted with amyotrophic lateral sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

    (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

89. A method of restoring motor function in a mammal afflicted with multiple sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

90. A method of restoring motor function in a mammal afflicted with a spinal cord injury, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

91. A method of preserving motor function in a mammal afflicted with or at risk of amyotrophic lateral sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

92. A method of preserving motor function in a mammal afflicted with or at risk of multiple sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

93. A method of preserving motor function in a mammal afflicted with or at risk of a spinal cord injury, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by Generic Sequence 7, SEQ ID NO: 4;

(d) defined by Generic Sequence 8, SEQ ID NO: 5;

(e) defined by Generic Sequence 9, SEQ ID NO: 6;

(f) defined by Generic Sequence 10, SEQ ID NO: 7, and

(g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

94. A method of treating amyotrophic lateral sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1,

human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

95. A method of treating multiple sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

96. A method of treating a spinal cord injury, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

97. A method of restoring motor function in a mammal afflicted with amyotrophic lateral sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

98. A method of restoring motor function in a mammal afflicted with multiple sclerosis, comprising the step of administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

99. A method of restoring motor function in a mammal afflicted with a spinal cord injury, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A,

BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

100. A method of preserving motor function in a mammal afflicted with or at risk of amyotrophic lateral sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

101. A method of preserving motor function in a mammal afflicted with or at risk of multiple sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

102. A method of preserving motor function in a mammal afflicted with or at risk of a spinal cord injury, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

103. The method of claim 82, 83, 84, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101 and 102, wherein said morphogen is complexed with at least one pro-domain polypeptide selected from the group consisting of the pro-domains of OP-1, OP-2, 60A, GDF-1, BMP-2A, BMP-2B, DPP, Vgl, Vgr-1, BMP-3, BMP-5, and BMP-6.

104. The method of claim 103, wherein said morphogen is complexed with a pair of said pro-domain polypeptides.--

REMARKS

Upon entry of the foregoing amendments, claims 82-104 are pending in the application. Claim 1 is cancelled in the present Preliminary Amendment. Claims 2-81 are cancelled in the Transmittal of Filing Under 37 C.F.R. 1.60(b). New claims 82-104 are fully supported by the Specification as originally filed.

Applicant respectfully submit that the present continuation application, as amended herein, should be in condition for allowance. If the Examiner believes that a telephone conversation with Applicant' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned attorney at (617) 248-7013.

Respectfully submitted,

Dated: September 25, 1997

Thomas C. Meyers
Reg. No. 36,989
Attorney for Applicants

TESTA, HURWITZ & THIBEAULT, LLP
High Street Tower
125 High Street
Boston, MA 02110
Tel: 617-248-7013
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389MM2054/39.413033_1

EXPRESS MAIL MAILING LABEL

EM354411625US

PATENT

Atty. Docket No.: CRP-070

COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

(Original, Design, National Stage of PCT, Supplemental, Divisional, Continuation or CIP)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: (check one applicable item below)

_X_ original

___ design

___ supplemental

___ national stage of PCT

___ divisional

___ continuation

___ continuation-in-part (CIP)

INVENTORSHIP IDENTIFICATION

My residence, post office address and citizenship are as stated next to my name, I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR

08/375,260

the specification of which (check one):

_X_ is attached hereto.

___ was filed on ________________ as Application Serial No. ________________ or

___ Express Mail No., as Serial No. not yet known ________________

and was amended on ________________ (if applicable).

___ was described and claimed in PCT International Application No. ________________ filed on ________________ and as amended under PCT Article 19 on ________________ (if any).

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose information which is material to the examination of this application with Title 37, Code of Federal Regulations, §1.56(a).

___ In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.97.

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Check one:

_X_ no such applications have been filed.

___ such applications have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (mo.,day, year)	Priority Claimed Under 37 USC 119
WO	PCT/US92/01968	March 11, 1992	___ YES NO X
			___ YES NO ___
			___ YES NO ___

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier United States application, if any, described below, I do not believe that the same was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to said earlier application, or in public use or on sale in the United States more than one year prior to said earlier application, that the said common subject matter has not been patented or made the subject of an inventor's certificate

issued before the date of said earlier application in any country foreign to the United States on an application, filed by me or my legal representatives or assigns more than twelve months prior to said application and that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

(Application Serial No.)	(Filing Date)	(Status)
753,059	August 30, 1991	pending
667,274	March 11, 1991	pending (patented, pending, aband.)
752,764	August 30, 1991	pending (patented, pending, aband.)

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the United States Patent and Trademark Office connected therewith:

Edmund R. Pitcher,	Reg. No. 27,829
Steven M. Bauer,	Reg. No. 31,481
Paula A. Campbell,	Reg. No. 32,503
Kathleen A. Williams,	Reg. No. 34,380
Robin D. Kelley,	Reg. No. 34,637
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Robert J. Tosti,	Reg. No. 35,393
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Direct correspondence to:

Patent Administrator
Creative BioMolecules, Inc.

35 South Street
Hopkinton, Massachusetts 01748 U.S.A.

Direct telephone calls to: Robin D. Kelley
(617) 248-7477

(name and telephone number)

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

08/469,325

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

David C. Rueger
Full name of sole or first inventor

U.S.A.
Citizenship

Inventor's signature

7/30/92
Date

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Residence

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Thangavel Kuberasampath
Full name of inventor

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Citizenship

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7/30/92
Date

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Residence

6 Spring Street/Medway, MA 02053
Post Office Address

CHECK PROPER BOX(ES) FOR ANY OF THE FOLLOWING ADDED PAGE(S) WHICH FORM A PART OF THIS DECLARATION

_x_ Signature for third and subsequent joint inventors. Number of pages added 2 .

___ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added ____.

___ Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. Number of pages added ____.

___ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation in-part (CIP) application. ___ Number of pages added.

___ Authorization of attorney(s) to accept and follow instructions from representative.

If no further pages form a part of this Declaration then end this Declaration with this page and check the following item.

___ This declaration ends this page.

ADDED PAGES FOR ADDITIONAL INVENTORS

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Full name of inventor

U.S.A.
Citizenship

Inventor's signature

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Engin Ozkaynak
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U.S.A.
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Roy H. L. Pang
Full name of inventor

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ADDED PAGES FOR ADDITIONAL INVENTORS

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Roy H. L. Pang
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Charles M. Cohen
Full name of inventor

U.S.A.
Citizenship

Inventor's signature

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98 Winthrop Street/Medway, MA 02053
Post Office Address

299RDK2054/39.BI8

08937755.092567

EXPRESS MAIL MAILING LABEL

NO. EM354411625US

PATENT

Attorney's Docket No. CRP-070

Applicant or Patentee: David C. Rueger et al.

Serial or Patent No.: 0 /

Filed or Issued: July 31, 1992

For: Morphogen-Induced Nerve Regeneration and Repair

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c))—SMALL BUSINESS CONCERN

I hereby declare that I am

☐ the owner of the small business concern identified below:

☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Creative BioMolecules, Inc.

ADDRESS OF CONCERN 35 South Street
Hopkinton, Massachusetts 01748

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I hereby declare that rights under contract or law have been conveyed, to and remain with the small business concern identified above with regard to the invention, entitled Morphogen-Induced Nerve Regeneration and Repair

by inventor(s) David C. Rueger, Thangavel Kuberasampath, Hermann Oppermann, Engin Ozkaynak, Roy H.L. Pang, Charles M. Cohen

described in

☒ the specification filed herewith.

☐ application serial no. 0 / ________, filed ________.

☐ patent no. ________, issued ________.

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SIGNATURE ______________ Date July 30, 1992

Morphogen-Induced Nerve Regeneration and Repair

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of USSN 752,764 and copending USSN 753,059, both filed August 30, 1991 and both continuations-in-part of USSN 667,274, filed March 11, 1991.

BACKGROUND OF THE INVENTION

The present invention relates to methods for enhancing the survival of neuronal cells *in vivo* and to methods, compositions and devices for maintaining neural pathways *in vivo*. More particularly, the invention provides methods for enhancing survival of neuronal cells at risk of dying, including methods for redifferentiating transformed cells of neural origin and methods for maintaining phenotypic expression of differentiated neuronal cells. The invention also provides means for repairing damaged neural pathways, including methods for stimulating axonal growth over extended distances, and methods for alleviating immunologically-related nerve tissue damage. In a particular embodiment of the invention, this invention provides a method for stimulating cell adhesion molecule expression in cells, and particularly nerve cell adhesion molecule expression in neurons. Finally, the invention provides means for evaluating nerve tissue stasis and identifying neural dysfunction in a mammal.

The mammalian nervous system comprises a peripheral nervous system (PNS) and a central nervous system (CNS, comprising the brain and spinal cord), and is composed of two principal classes of cells: neurons and glial cells. The glial cells fill the spaces between neurons, nourishing them and modulating their function. Certain glial cells, such as Schwann cells in the PNS and oligodendrocytes in the CNS, also provide a protective myelin sheath that surrounds and protects neuronal axons, which are the processes that extend from the neuron cell body and through which the electric impulses of the neuron are transported. In the peripheral nervous system, the long axons of multiple neurons are bundled together to form a nerve or nerve fiber. These, in turn, may be combined into fascicles, wherein the nerve fibers form bundles embedded, together with the intraneural vascular supply, in a loose collagenous matrix bounded by a protective multilamellar sheath. In the central nervous system, the neuron cell bodies are visually distinguishable from their myelin-ensheathed processes, and are referenced in the art as grey and white matter, respectively.

During development, differentiating neurons from the central and peripheral nervous systems send out axons that must grow and make contact with specific target cells. In some cases, growing axons must cover enormous distances; some grow into the periphery, whereas others stay confined within the central nervous system. In mammals, this stage of neurogenesis is complete during the embryonic phase of life and neuronal cells do not multiply once they have fully differentiated.

Accordingly, the neural pathways of a mammal are particularly at risk if neurons are subjected to mechanical or chemical trauma or to neuropathic degeneration sufficient to put the neurons that define the pathway at risk of dying. A host of neuropathies, some of which affect only a subpopulation or a system of neurons in the peripheral or central nervous systems have been identified to date. The neuropathies, which may affect the neurons themselves or the associated glial cells, may result from cellular metabolic dysfunction, infection, exposure to toxic agents, autoimmunity dysfunction, malnutrition or ischemia. In some cases the cellular dysfunction is thought to induce cell death directly. In other cases, the neuropathy may induce sufficient tissue necrosis to stimulate the body's immune/inflammatory system and the mechanisms of the body's immune response to the initial neural injury then destroys the neurons and the pathway defined by these neurons.

Currently no satisfactory method exists to repair the damage caused by these neuropathies, which include multiple sclerosis, amyotrophic lateral sclerosis (ALS), Huntington's chorea, Alzheimer's disease, Parkinson's disease (parkinsonism), and metabolically derived disorders, such as hepatic encephalopathy. Current attempts to counteract the effects of severe traumatic or neural degenerative lesions of the brain and/or spinal cord have to date primarily involved implantation of embryonic neurons in an effort to replace functionally, or otherwise compensate for, lost or deficient neurons. Currently, however, human fetal cell transplantation research is severely restricted. Administration of neurotrophic factors such as nerve growth factor and insulin-like growth factor also have

been suggested to stimulate neuronal growth within the CNS. (See, for example, Lundborg, (1987) Acta Orthop. Scand. 58:145-169 and US Pat. No. 5,093,317.) Administration of neurotrophic factors to the CNS requires bypassing the blood-brain barrier. The barrier may be overcome by direct infusion, or by modifying the molecule to enhance its transport across the barrier, as by chemical modification or conjugation, or by molecule truncation. Schwann cells also have been grafted to a site of a CNS lesion in an attempt to stimulate and maintain growth of damaged neuronal processes (Paino et al. (1991) Exp. Neurology 114(2):254-257).

Where the damaged neural pathway results from CNS axonal damage, autologous peripheral nerve grafts have been used to bridge lesions in the central nervous system and to allow axons to make it back to their normal target area. In contrast to CNS neurons, neurons of the peripheral nervous system can extend new peripheral processes in response to axonal damage. This regenerative property of peripheral nervous system axons is thought to be sufficient to allow grafting of these segments to CNS axons. Successful grafting appears to be limited, however, by a number of factors, including the length of the CNS axonal lesion to be bypassed, and the distance of the graft sites from the CNS neuronal cell bodies, with successful grafts occurring near the cell body.

Within the peripheral nervous system, this cellular regenerative property of neurons has limited ability to repair function to a damaged neural pathway. Specifically, the new axons extend randomly, and are often misdirected, making contact with inappropriate

targets that can cause abnormal function. For example, if a motor nerve is damaged, regrowing axons may contact the wrong muscles, resulting in paralysis. In addition, where severed nerve processes result in a gap of longer than a few millimeters, e.g., greater than 10 millimeters (mm), appropriate nerve regeneration does not occur, either because the processes fail to grow the necessary distance, or because of misdirected axonal growth. Efforts to repair peripheral nerve damage by surgical means has met with mixed results, particularly where damage extends over a significant distance. In some cases, the suturing steps used to obtain proper alignment of severed nerve ends stimulates the formulation of scar tissue which is thought to inhibit axon regeneration. Even where scar tissue formation has been reduced, as with the use of nerve guidance channels or other tubular prostheses, successful regeneration generally still is limited to nerve damage of less than 10 millimeters in distance. In addition, the reparative ability of peripheral neurons is significantly inhibited where an injury or neuropathy affects the cell body itself or results in extensive degeneration of a distal axon.

Mammalian neural pathways also are at risk due to damage caused by neoplastic lesions. Neoplasias of both the neurons and glial cells have been identified. Transformed cells of neural origin generally lose their ability to behave as normal differentiated cells and can destroy neural pathways by loss of function. In addition, the proliferating tumors may induce lesions by distorting normal nerve tissue structure, inhibiting pathways by compressing nerves, inhibiting cerbrospinal

fluid or blood supply flow, and/or by stimulating the body's immune response. Metastatic tumors, which are a significant cause of neoplastic lesions in the brain and spinal cord, also similarly may damage neural pathways and induce neuronal cell death.

One type of morphoregulatory molecule associated with neuronal cell growth, differentiation and development is the cell adhesion molecule ("CAM"), most notably the nerve cell adhesion molecule (N-CAM). CAMs belong to the immunoglobulin super-family and mediate cell-cell interactions in developing and adult tissues through homophilic binding, i.e., CAM-CAM binding on apposing cells. A number of different CAMs currently have been identified. Of these, the most thoroughly studied to date are N-CAM and L-CAM (liver cell adhesion molecules), both of which have been identified on all cells at early stages of development, as well as in different adult tissues. In neural tissue development, N-CAM expression is believed to be important in tissue organization, neuronal migration, nerve-muscle tissue adhesion, retinal formation, synaptogenesis, and neural degeneration. Reduced N-CAM expression also is thought to be associated with nerve dysfunction. For example, expression of at least one form of N-CAM, N-CAM-180, is reduced in a mouse dysmyelinating mutant (Bhat (1988) Brain Res. 452:373-377). Reduced levels of N-CAM also have been associated with normal pressure hydrocephalus (Werdelin (1989) Acta Neurol. Scand. 79:177-181), and with type II schizophrenia (Lyons et al., (1988) Biol. Psychiatry 23:769-775.) In addition, antibodies to N-CAM have been shown to disrupt functional recovery in injured nerves (Remsen (1990) Exp. Neurobiol. 110:268-273).

It is an object of this invention to provide methods for enhancing survival of neurons at risk of dying in a mammal. Another object is to provide methods for maintaining neural pathways in vivo at risk of injury, or following damage to nerve tissue due to mechanical or chemical trauma, a neuropathy, or a neoplastic lesion. Another object is to provide compositions and devices for repairing gaps in a neural pathway of the peripheral nervous system. Yet another object is to provide a means for redifferentiating transformed cells defining neural pathways, particularly transformed cells of neural origin. Another object is to provide a means for stimulating CAM expression, particularly N-CAM expression in a cell. Yet another object is to provide methods for monitoring the status of nerve tissue by monitoring fluctuations in protein levels present in nerve tissue, serum and/or cerebrospinal fluid. These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Summary of the Invention

The present invention provides methods and compositions for maintaining neural pathways in a mammal in vivo, including methods for enhancing the survival of neural cells.

In one aspect, the invention features compositions and therapeutic treatment methods that comprise the step of administering to a mammal a therapeutically effective amount of a morphogenic protein ("morphogen"), as defined herein, upon injury to a neural pathway, or in anticipation of such injury, for a time and at a concentration sufficient to maintain the neural pathway, including repairing damaged pathways, or inhibiting additional damage thereto.

In another aspect, the invention features compositions and therapeutic treatment methods for maintaining neural pathways in a mammal in vivo which include administering to the mammal, upon injury to a neural pathway or in anticipation of such injury, a compound that stimulates in vivo a therapeutically effective concentration of an endogenous morphogen within the body of the mammal sufficient to maintain the neural pathway, including repairing damaged pathways or inhibiting additional damage thereto. These compounds are referred to herein as morphogen-stimulating agents, and are understood to include substances which, when administered to a mammal, act on

tissue(s) or organ(s) that normally are responsible for, or capable of, producing a morphogen and/or secreting a morphogen, and which cause the endogenous level of the morphogen to be altered. The agent may act, for example, by stimulating expression and/or secretion of an endogenous morphogen.

In particular, the invention provides methods for enhancing the survival of neurons at risk of dying, including protecting neurons from the tissue destructive effects associated with the body's immune/ inflammatory response to a nerve injury. The invention also provides methods for stimulating neurons to maintain their differentiated phenotype, including inducing the redifferentiation of transformed cells of neuronal origin to a morphology characteristic of untransformed neurons. In one embodiment, the invention provides means for stimulating production of cell adhesion molecules in cells, particularly nerve cell adhesion molecules (N-CAM) in neurons. The invention also provides methods, compositions and devices for stimulating cellular repair of damaged neurons and neural pathways, including regenerating damaged axons of the peripheral and central nervous systems. In addition, the invention also provides means for evaluating the status of nerve tissue, and for detecting and monitoring neuropathies in a mammal by monitoring fluctuations in the morphogen levels or endogenous morphogen antibody levels present in a mammal's serum or cerebrospinal fluid.

As used herein, a "neural pathway" describes a nerve circuit for the passage of electric signals from a source to a target cell site. The pathway includes the neurons through which the electric impulse is

transported, including groups of interconnecting neurons, the nerve fibers formed by bundled neuronal axons, and the glial cells surrounding and associated with the neurons.

In one aspect of the invention, the morphogens described herein are useful in repairing damaged neural pathways of the peripheral nervous system. In particular, the morphogens are useful for repairing damaged pathways, including transected or otherwise damaged nerve fibers (nerves) requiring regeneration of neuronal processes, particularly axons, over extended distances to bridge a gap in the nerve itself, or between the nerve and a post-synaptic cell. Specifically, the morphogens described herein are capable of stimulating complete axonal nerve regeneration, including vascularization and reformation of the protective myelin sheath. The morphogen preferably is provided to the site of injury dispersed in a biocompatible, bioresorbable carrier material capable of maintaining the morphogen at the site and, where necessary, means for directing axonal growth from the proximal to the distal ends of a severed neuron or nerve. For example, means for directing axonal growth may be required where nerve regeneration is to be induced over an extended distance, such as greater than 10 mm. Many carriers capable of providing these functions are envisioned. For example, useful carriers include substantially insoluble materials or viscous solutions prepared as disclosed herein comprising laminin, hyaluronic acid or collagen, or other suitable synthetic, biocompatible polymeric materials such as

polylactic, polyglycolic or polybutyric acids and/or copolymers thereof. The currently preferred carrier comprises an extracellular matrix composition, such as one described herein derived, for example, from mouse sarcoma cells. Also envisioned as especially useful are brain tissue-derived extracellular matrices.

In a particularly preferred embodiment, the morphogen is provided to the site as part of a device wherein the morphogen is disposed in a nerve guidance channel which spans the distance of the damaged pathway. The channel acts both as a protective covering and a physical means for guiding growth of a neuronal process such as an axon. Useful channels comprise a biocompatible membrane or casing, which may be tubular in structure, having a dimension sufficient to span the gap or break in the nerve to be repaired, and having openings adapted to receive severed nerve ends. The casing or membrane may be made of any biocompatible, nonirritating material, such as silicone or a biocompatible polymer such as polyethylene or polyethylene vinyl acetate. The casing also may be composed of biocompatible, bioresorbable polymers, including, for example, collagen, hyaluronic acid, polylactic, polybutyric and polyglycolic acids. In a currently preferred embodiment, the outer surface of the channel is substantially impermeable.

The morphogen may be disposed in the channel in association with a biocompatible carrier material, or it may be adsorbed to or otherwise associated with the inner surface of the casing, such as is described in U.S. Pat. No. 5,011,486, provided that the morphogen is accessible to the severed nerve ends. Additionally, although the nerve guidance channels described herein

generally are tubular in shape, it should be evident to those skilled in the art that various alternative shapes may be employed. The lumen of the guidance channels may, for example, be oval or even square in cross section. Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may be secured to the nerve guidance channels by means of sutures, biocompatible adhesives such as fibrin glue, or other means known in the medical art.

The morphogens described herein also are envisioned to be useful in autologous peripheral nerve segment implants to bypass damaged neural pathways in the central nervous system, such as in the repair of damaged or detached retinas, or other damage to the optic nerve. Here the morphogen is provided to the site of attachment to stimulate axonal growth at the graft site, particularly where the damaged axonal segment to be bypassed occurs far from the neuronal cell body.

The morphogens described herein also are useful for enhancing survival of neuronal cells at risk of dying, thereby preventing, limiting or otherwise inhibiting damage to neural pathways. Non-mitotic neurons are at risk of dying as a result of a neuropathy or other cellular dysfunction of a neuron or glial cell inducing cell death, or following a chemical or mechanical lesion to the cell or its surrounding tissue. The chemical lesions may result from known toxic agents, including lead, ethanol, ammonia, formaldehyde and many other organic solvents, as well as the toxins in cigarette smoke and opiates. Excitatory amino acids, such as glutamate also may play a role in the

pathogenesis of neuronal cell death (see Freese et al. (1990) Brain Res. 521:254-264). Neuronal cell death also is thought to be a significant contributing factor in a number of neurodegenerative diseases, including Alzheimer's disease, Huntington's chorea, and Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis. The etiology of these neuropathies may be metabolic, as results in hepatic encephalopathy, infectious, toxic, autoimmune, nutritional or ischemic. In addition, ethanol and a number of other toxins also have been identified as significant contributing factors in neurodegenerative diseases. The morphogens described herein may be provided to cells at risk of dying to enhance their survival and thereby protect the integrity of the neural pathway. The morphogens may be provided directly to the site, or they may be provided systemically. Alternatively, as described above, an agent capable of stimulating endogenous morphogen expression and/or secretion, preferably in cells associated with the nerve tissue of interest, may be administered to the mammal.

In another aspect of the invention, the method disclosed is useful for redifferentiating transformed cells, particularly transformed cells of neuronal or glial origin, such that the morphogen-treated cells are induced to display a morphology characteristic of untransformed cells. Where the transformed cells are cells of neuronal origin, morphogen treatment preferably induces cell rounding and cell aggregation (clumping), cell-cell adhesion, neurite outgrowth formation and elongation, and N-CAM production. The methods described herein are anticipated to substantially inhibit or reduce neural cell tumor formation and/or proliferation in nerve tissue. It is

anticipated that the methods of this invention will be useful in substantially reducing the effects of various carcinomas of nerve tissue origin such as retinoblastomas, neuroblastomas, and gliomas or glioblastomas. In addition, the method also is anticipated to aid in inhibiting neoplastic lesions caused by metastatic tissue. Metastatic tumors are one of the most common neoplasms of the CNS, as they can reach the intracranial compartment through the bloodstream. Metastatic tumors may damage neural pathways for example, by distorting normal nerve tissue structure, compressing nerves, blocking flow of cerebrospinal fluid or the blood supply nourishing brain tissue, and/or by stimulating the body's immune response.

In another aspect of the invention, the morphogens described herein are useful for providing neuroprotective effects to alleviate neural pathway damage associated with the body's immune/inflammatory response to an initial injury to nerve tissue. Such a response may follow trauma to nerve tissue, caused, for example, by an autoimmune dysfunction, neoplastic lesion, infection, chemical or mechanical trauma, disease, by interruption of blood flow to the neurons or glial cells, for example following ischemia or hypoxia, or by other trauma to the nerve or surrounding material. For example, the primary damage resulting from hypoxia or ischemia-reperfusion following occlusion of a neural blood supply, as in an embolic stroke, is believed to be immunologically associated. In addition, at least part of the damage associated with a number of primary brain tumors also appears to be immunologically related. Application of the morphogen directly to the cells to be treated, or

providing the morphogen to the mammal systemically, for example, intravenously or indirectly by oral administration, may be used to alleviate and/or inhibit the immunologically related response to a neural injury. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion <u>in vivo</u>, preferably at the site of injury, also may be used. Where the injury is to be induced, as during surgery or other aggressive clinical treatment, the morphogen or agent may be provided prior to induction of the injury to provide a neuroprotective effect to the nerve tissue at risk.

In still another aspect, the invention described herein provides methods for supporting the growth and maintenance of differentiated neurons, including inducing neurons to continue expressing their phenotype. It is anticipated that this activity will be particularly useful in the treatment of nerve tissue disorders where loss of function is caused by reduced or lost cellular metabolic function and cells become senesent or quiescent, such as is thought to occur in aging cells and to be manifested in Alzheimer's disease. Application of the morphogen directly to cells to be treated, or providing it systemically by parenteral or oral administration stimulates these cells to continue expressing their phenotype, significantly inhibiting and/or reversing the effects of the cellular metabolic dysfunction, thereby maintaining the neural pathway at risk. Alternatively, administration of an agent capable of stimulating endogenous morphogen expression and/or secretion <u>in vivo</u> may be used.

In still another aspect, the invention provides methods for stimulating CAM expression levels in a cell, particularly N-CAM expression in neurons. CAMs are molecules defined as carrying out cell-cell interactions necessary for tissue formation. CAMs are believed to play a fundamental regulatory role in tissue development, including tissue boundary formation, embryonic induction and migration, and tissue stabilization and regeneration. Altered CAM levels have been implicated in a number of tissue disorders, including congenital defects, neoplasias, and degenerative diseases.

In particular, N-CAM expression is associated with normal neuronal cell development and differentiation, including retinal formation, synaptogenesis, and nerve-muscle tissue adhesion. Inhibition of one or more of the N-CAM isoforms is known to prevent proper tissue development. Altered N-CAM expression levels also are associated with neoplasias, including neuroblastomas (see infra), as well as with a number of neuropathies, including normal pressure hydrocephalous and type II schizophrenia. Application of the morphogen directly to the cells to be treated, or providing the morphogen to the mammal systemically, for example, parenterally, or indirectly by oral administration, may be used to induce cellular expression of one or more CAMs, particularly N-CAMs. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion in vivo, preferably at the site of injury, also may be used to induce CAM production.

CAMs also have been postulated as part of a morphoregulatory pathway whose activity is induced by a to date unidentified molecule (See, for example,

Edelman, G.M. (1986) Ann. Rev. Cell Biol. 2:81-116). Without being limited to any given theory, the morphogens described herein may act as the inducer of this pathway.

Finally, modulations of endogenous morphogen levels may be monitored as part of a method of detecting nerve tissue dysfunction. Specifically, modulations in endogenous morphogen levels are anticipated to reflect changes in nerve tissue status. Morphogen expression may be monitored directly in biopsied cell samples, in cerebrospinal fluid, or serum. Alternatively, morphogen levels may be assessed by detecting changes in the levels of endogenous antibodies to the morphogen. For example, one may obtain serum samples from a mammal, and then detect the concentration of morphogen or antibody present in the fluid by standard protein detection means known to those skilled in the art. As an example, binding protein capable of interacting specifically with the morphogen of interest such as an anti-morphogen antibody may be used to detect a morphogen in a standard immunoassay. The morphogen levels detected then may be compared to a previously determined standard or reference level, with changes in the detected levels being indicative of the status of the tissue.

In one preferred embodiment of the invention, the morphogen or morphogen-stimulating agent is administered systemically to the individual, e.g., orally or parenterally. In another embodiment of the invention, the morphogen may be provided directly to the nerve tissue, e.g., by injection to the cerebral spinal fluid or to a nerve tissue locus.

In any treatment method of the invention, "administration of morphogen" refers to the administration of the morphogen, either alone or in combination with other molecules. For example, the mature form of the morphogen may be provided in association with its precursor "pro" domain, which is known to enhance the solubility of the protein. Other useful molecules known to enhance protein solubility include casein and other milk components, as well as various serum proteins. Additional useful molecules which may be associated with the morphogen or morphogen-stimulating agent include tissue targeting molecules capable of directing the morphogen or morphogen-stimulating agent to nerve tissue. Tissue targeting molecules envisioned to be useful in the treatment protocols of this invention include antibodies, antibody fragments or other binding proteins which interact specifically with surface molecules on nerve tissue cells.

Still another useful tissue targeting molecule is part or all of the morphogen precursor "pro" domain, particularly that of OP-1 or GDF-1. These proteins are found naturally associated with nerve tissue but also may be synthesized in other tissues and targeted to nerve tissue after secretion from the synthesizing tissue. For example, while the protein has been shown to be active in bone tissue, the primary source of OP-1 synthesis appears to be the tissue of the urogenic system (e.g., renal and bladder tissue), with secondary expression levels occurring in the brain, heart and lungs (see below.) Moreover, the protein has been

identified in serum, saliva and various milk forms. In addition, the secreted form of the protein comprises the mature dimer in association with the pro domain of the intact morphogen sequence. Accordingly, the associated morphogen pro domains may act to target specific morphogens to different tissues in vivo.

Associated tissue targeting or solubility-enhancing molecules also may be covalently linked to the morphogen using standard chemical means, including acid-labile linkages, which likely will be preferentially cleaved in the acidic environment of bone remodeling sites.

Finally, the morphogens or morphogen-stimulating agents provided herein also may be administered in combination with other molecules known to be beneficial in maintaining neural pathways, including, for example, nerve growth factors and anti-inflammatory agents.

Where the morphogen is intended for use as a therapeutic for disorders of the CNS, an additional problem must be addressed: overcoming the so-called "blood-brain barrier", the brain capillary wall structure that effectively screens out all but selected categories of molecules present in the blood, preventing their passage into the brain. The blood-brain barrier may be bypassed effectively by direct infusion of the morphogen or morphogen-stimulating agent into the brain. Alternatively, the morphogen or morphogen-stimulating agent may be modified to enhance its transport across the blood-brain barrier. For example, truncated forms of the morphogen or a morphogen-stimulating agent may be most successful. Alternatively, the morphogen or

morphogen-stimulating agent may be modified to render it more lipophilic, or it may be conjugated to another molecule which is naturally transported across the barrier, using standard means known to those skilled in the art, as, for example, described in Pardridge, Endocrine Reviews 7:314-330 (1986) and U.S. Pat. No. 4,801,575.

Accordingly, as used herein, a functional "analog" of a morphogen refers to a protein having morphogenic biological activity but possessing additional structural differences compared to a morphogen as defined herein, e.g., having additional chemical moieties not normally a part of a morphogen. Such moieties (introduced, for example, by acylation, alkylation, cationization, or glycosylation reactions, or other means for conjugating the moiety to the morphogen) may improve the molecule's solubility, absorption, biological half-life, or transport, e.g., across the blood-brain barrier.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from Drosophila), Vgl (from Xenopus), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos.5-14), and the recently identified 60A protein (from Drosophila, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF-β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are translated as a

precursor, having an N-terminal signal peptide sequence, typically less tahn about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

TABLE I

"OP-1"	Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined

by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

"OP-2" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)

"CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

"DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

"Vgl(fx)" refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in

Weeks (1987) Cell 51: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

"Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.

"GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 32. The prodomain likely extends from the signal peptide clavage site to residue 214; the mature protein likely is defined by residues 215-372.

"60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to

the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.) The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.

"BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) <u>Science</u> <u>242</u>: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.

"BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) <u>PNAS</u> <u>87</u>: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.

"BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appear sin Celeste, et al.

(1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of

acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. In addition, it is also anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer, α-amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N-terminus:

```
Cys Xaa Xaa Xaa Xaa  (Seq. ID No. 15)
 1               5
```

Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31), listed below. These

Generic Sequences accommodate the homologies shared among the various preferred members of this morphogen family identified in Table II, as well as the amino acid sequence variation among them. Specifically, Generic Sequences 3 and 4 are composite amino acid sequences of the following proteins presented in Table II and identified in Seq. ID Nos. 5-14: human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14.) The generic sequences include both the amino acid identity shared by the sequences in Table II, as well as alternative residues for the variable positions within the sequence. Note that these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids which influence the tertiary structure of the proteins.

<u>Generic Sequence 3</u>

```
          Leu Tyr Val Xaa Phe
          1               5

     Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                     10

     Xaa Ala Pro Xaa Gly Xaa Xaa Ala
      15                 20
```

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Gly Cys Xaa
95

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn); Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 =

(Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 = (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His or Asn); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or

Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or Arg);

<u>Generic Sequence 4</u>

```
Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
 1               5                   10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                 15
Xaa Ala Pro Xaa Gly Xaa Xaa Ala
 20                  25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
         30                  35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                 40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
         45                  50
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                 55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
     60                  65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
             70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 75                  80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
             85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 90                  95
Xaa Cys Gly Cys Xaa
        100
```

wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 = (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln); Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 = (Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp);

Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa at res.102 = (His or Arg).

Similarly, Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31) accommodate the homologies shared among all the morphogen protein family members identified in Table II. Specifically, Generic Sequences 5 and 6 are composite amino acid sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14), human BMP3 (Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq. ID Nos. 24-25). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeltons (Generic Sequences 5 and 6, respectively), as well as alternative residues for the variable positions within the sequence. As for Generic

Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

<u>Generic Sequence 5</u>

```
            Leu Xaa Xaa Xaa Phe
            1               5
        Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                        10
        Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
         15                 20
        Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                25                  30
        Xaa Pro Xaa Xaa Xaa Xaa Xaa
                        35
        Xaa Xaa Xaa Asn His Ala Xaa Xaa
                40                  45
        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                        50
        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
            55                  60
        Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
                    65
```

Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Xaa Cys Xaa
95

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.2 = (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu

or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln, His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

Generic Sequence 6

```
Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
 1               5                  10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                15
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
 20                  25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        30                  35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
        45                  50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
     60                  65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
            70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 75                  80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
            85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 90                  95
Xaa Cys Xaa Cys Xaa
        100
```

wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at

res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 = (Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln, Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53 = (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or

Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr, Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile, Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser); Xaa at res.100 = (Gly or Ala); and Xaa at res.102 = (His or Arg).

Particularly useful sequences for use as morphogens in this invention include the C-terminal domains, e.g., the C-terminal 96-102 amino acid residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A, CBMP-2B, GDF-1 (see Table II, below, and Seq. ID Nos. 5-14), as well as proteins comprising the C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see Seq. ID Nos. 24-28), all of which include at least the conserved six or seven cysteine skeleton. In addition, biosynthetic constructs designed from the generic sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S. Pat. No. 5,011,691, also are useful. Other sequences include the inhibins/activin proteins (see, for example, U.S. Pat. Nos. 4,968,590 and 5,011,691). Accordingly, other useful sequences are those sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity

with any of the sequences above. These are anticipated to include allelic and species variants and mutants, and biosynthetic muteins, as well as novel members of this morphogenic family of proteins. Particularly envisioned in the family of related proteins are those proteins exhibiting morphogenic activity and wherein the amino acid changes from the preferred sequences include conservative changes, e.g., those as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol. 5, Suppl. 3, pp. 345-362, (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington, D.C. 1979). As used herein, potentially useful sequences are aligned with a known morphogen sequence using the method of Needleman et al. ((1970) J.Mol.Biol. 48:443-453) and identities calculated by the Align program (DNAstar, Inc.). "Homology" or "similarity" as used herein includes allowed conservative changes as defined by Dayoff et al.

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in another preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 29).

The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include *E. coli* or mammalian cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods,

compositions and devices of this invention is disclosed in copending US patent application Serial Nos. 752,764, filed August 30, 1991, and 667,274, filed March 11, 1991, the disclosure of which are incorporated herein by reference.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both procaryotes and eucaryotes, to produce large quantities of active proteins capable of maintaining neural pathways in a mammal, including enhancing the survival of neurons at risk of dying and stimulating nerve regeneration and repair in a variety of mammals, including humans.

The foregoing and other objects, features and advantages of the present invention will be made more apparent from the following detailed description of the invention.

Brief Description of the Drawings:

The foregoing and other objects and features of this invention, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

Fig. 1(A and B) are photomicrographs illustrating the ability of morphogen (OP-1) to induce transformed neuroblastoma x glioma cells (1A) to redifferentiate to a morphology characteristic of untransformed neurons (1B);

Fig. 2A is a dose response curve for the induction of the 180 kDa and 140 kDa N-CAM isoforms in morphogen-treated NG108-15 cells;

Fig. 2B is a photomicrograph of a Western blot of whole cell extracts from morphogen-treated NG108-15 cells with an N-CAM-specific antibody; and

Fig. 3 is a plot of the mean number of cell aggregates counted in 20 randomly selected fields as a function of morphogen concentration.

Fig. 4 is a photomicrograph of an immunoblot demonstrating the presence of OP-1 in human serum.

Detailed Description of the Invention

It now has been discovered that the proteins described herein are effective agents for enhancing the survival of neurons, particularly neurons at risk of dying, and for maintaining neural pathways in a mammal. As described herein, these proteins ("morphogens") are capable of enhancing survival of non-mitotic neurons, stimulating neuronal CAM expression, maintaining the phenotypic expression of differentiated neurons, inducing the redifferentiation of transformed cells of neural origin, and stimulating axonal growth over breaks in neural processes, particularly large gaps in distal axons. The proteins also are capable of providing a neuroprotective effect to alleviate the tissue destructive effects associated with immunologically-related nerve tissue damage. Finally, the proteins may be used as part of a method for monitoring the viability of nerve tissue in a mammal.

Provided below are detailed descriptions of suitable morphogens useful in the methods, compositions and devices of this invention, as well as methods for their administration and application, and numerous, nonlimiting examples which 1) illustrate the suitability of the morphogens and morphogen-stimulating agents described herein as therapeutic agents for maintaining nerual pathways in a mammal and enhancing survival of neuronal cells at risk of dying; and 2) provide assays with which to test candidate morphogens and morphogen-stimulating agents for their efficacy.

I. Useful Morphogens

As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see supra). Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. Details of how the morphogens useful in the method of this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in USSN 667,274, filed March 11, 1991 and USSN 752,764, filed August 30, 1991, the disclosures of which are hereby incorporated by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from procaryotic or eucaryotic host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Particularly useful proteins include those which comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic

constructs such as those disclosed in U.S. Pat. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

Accordingly, the morphogens useful in the methods and compositions of this invention also may be described by morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with any of the sequences described above, where "homology" is as defined herein above.

The morphogens useful in the method of this invention also can be described by any of the 6 generic sequences described herein (Generic Sequences 1, 2, 3, 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

```
Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
 1               5
```

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33), 60A protein (from Drosophila, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method

of Needleman et al. (1970) J. Mol. Biol., 48:443-453, calculated using the Align Program (DNAstar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

TABLE II

hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
mOP-1	...	...	...	...	...	...	...	...
hOP-2	...	Arg	Arg	...	...	...	...	...
mOP-2	...	Arg	Arg	...	...	...	...	...
DPP	...	Arg	Arg	...	Ser	...	...	...
Vgl	...	...	Lys	Arg	His	...	...	...
Vgr-1	...	...	...	...	Gly	...	...	...
CBMP-2A	...	...	Arg	...	Pro	...	...	...
CBMP-2B	...	Arg	Arg	...	Ser	...	...	...
BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...
GDF-1	...	Arg	Ala	Arg	Arg	...	...	...
60A	...	Gln	Met	Glu	Thr	...	...	...
BMP5	...	...	...	...	...	...	...	...
BMP6	...	Arg	...	...	...	...	...	...
	1				5			

hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP-1	...	...	...	...	...	...	...	...	...

hOP-2	...	...	Gln	...	...	...	...	Leu	...
mOP-2	Ser	...	...	...	...	...	...	Leu	...
DPP	Asp	...	Ser	...	Val	...	...	Asp	...
Vgl	Glu	...	Lys	...	Val	...	...	...	Asn
Vgr-1	...	...	Gln	...	Val	...	...	...	...
CBMP-2A	Asp	...	Ser	...	Val	...	...	Asn	...
CBMP-2B	Asp	...	Ser	...	Val	...	...	Asn	...
BMP3	Asp	...	Ala	...	Ile	...	...	Ser	Glu
GDF-1	...	...	...	Glu	Val	...	...	His	Arg
60A	Asp	...	Lys	...	...	...	...	His	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Gln	...	...	...	...	...	...
		10					15		

hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Val	...	...	...	Gln	...	...	Ser
mOP-2	...	Val	...	...	...	Gln	...	...	Ser
DPP	...	...	Val	...	...	Leu	...	...	Asp
Vgl	...	Val	...	...	...	Gln	...	...	Met
Vgr-1	...	...	...	...	...	Lys	...	...	...
CBMP-2A	...	...	Val	...	...	Pro	...	...	His
CBMP-2B	...	...	Val	...	...	Pro	...	...	Gln
BMP3	...	...	...	Ser	...	Lys	Ser	Phe	Asp
GDF-1	...	Val	...	...	...	Arg	...	Phe	Leu
60A	...	...	...	...	...	...	...	...	Gly
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	Lys	...	...	...
			20					25	

hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	...	...	...	...	...	Ser
mOP-2	...	...	...	...	...	...	...	...	...

DPP	...	...	...	...	His	...	Lys	...	Pro
Vgl	...	Asn	...	...	Tyr	...	...	...	Pro
Vgr-1	...	Asn	...	...	Asp	...	...	...	Ser
CBMP-2A	...	Phe	...	...	His	...	Glu	...	Pro
CBMP-2B	...	Phe	...	...	His	...	Asp	...	Pro
BMP3	...	...	...	...	Ser	...	Ala	...	Gln
GDF-1	...	Asn	...	...	Gln	...	Gln	...	...
60A	...	Phe	...	...	Ser	...	...	...	Asn
BMP5	...	Phe	...	...	Asp	...	...	...	Ser
BMP6	...	Asn	...	...	Asp	...	...	...	Ser
				30					35

hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	Asp	...	Cys	...	...	...
mOP-2	...	...	...	Asp	...	Cys	...	...	...
DPP	...	...	...	Ala	Asp	His	Phe	...	Ser
Vgl	Tyr	...	...	Thr	Glu	Ile	Leu	...	Gly
Vgr-1	...	...	...	...	Ala	His	...	...	...
CBMP-2A	...	...	...	Ala	Asp	His	Leu	...	Ser
CBMP-2B	...	...	...	Ala	Asp	His	Leu	...	Ser
GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...
BMP3	...	...	Met	Pro	Lys	Ser	Leu	Lys	Pro
60A	...	...	...	...	Ala	His	...	...	...
BMP5	...	...	...	...	Ala	His	Met	...	...
BMP6	...	...	...	...	Ala	His	Met	...	...
					40				

hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	...	...	Leu	...	Ser	...
mOP-2	...	...	...	...	...	Leu	...	Ser	...
DPP	...	...	...	...	Val	...	...	...	...
Vgl	Ser	...	...	...	...	Leu	...	...	...
Vgr-1	...	...	...	...	...	...	...	...	...

CBMP-2A	...	...	...	...	...	...	...	...	...
CBMP-2B	...	...	...	...	...	...	...	...	...
BMP3	Ser	...	...	...	Thr	Ile	...	Ser	Ile
GDF-1	Leu	...	...	...	Val	Leu	Arg	Ala	...
60A	...	...	...	...	...	...	...	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
	45					50			

hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP-1	...	...	...	...	...	...	Asp	...	...
hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
DPP	...	Asn	Asn	Asn	...	...	Gly	Lys	...
Vgl	...	...	Ser	...	Glu	...	...	Asp	Ile
Vgr-1	...	...	Val	Met	...	...	...	Tyr	...
CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
60A	...	...	Leu	Leu	Glu	...	Lys	Lys	...
BMP5	...	...	Leu	Met	Phe	...	Asp	His	...
BMP6	...	...	Leu	Met	...	...	...	Tyr	...
		55					60		

hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	Ala	...	...	...	...	...	Lys
mOP-2	...	...	Ala	...	...	...	...	...	Lys
DPP	...	...	Ala	...	...	Val	...	...	...
Vgl	...	Leu	...	...	...	Val	...	...	Lys
Vgr-1	...	...	...	...	...	...	...	...	Lys
CBMP-2A	...	...	Ala	...	...	Val	...	...	Glu

CBMP-2B	...	...	Ala	...	...	Val	...	...	Glu
BMP3	...	Glu	...	...	...	Val	...	Glu	Lys
GDF-1	Asp	Leu	...	...	...	Val	...	Ala	Arg
60A	...	...	...	...	...	...	...	...	Arg
BMP5	...	...	...	...	...	...	...	...	Lys
BMP6	...	...	...	...	...	...	...	...	Lys
			65					70	

hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
mOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
Vgl	Met	Ser	Pro	...	...	Met	...	Phe	Tyr
Vgr-1	Val	...	...	...	...	...	...	...	...
DPP	...	Asp	Ser	Val	Ala	Met	...	...	Leu
CBMP-2A	...	Ser	...	...	...	Met	...	...	Leu
CBMP-2B	...	Ser	...	...	...	Met	...	...	Leu
BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
GDF-1	...	Ser	Pro	...	...	...	...	Phe	...
60A	...	Gly	...	Leu	Pro	...	...	...	His
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
				75					80

hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-2	...	Ser	...	Asn	...	...	...	...	Arg
DPP	Asn	...	Gln	...	Thr	...	Val	...	...
Vgl	...	Asn	Asn	Asp	...	...	Val	...	Arg
Vgr-1	...	...	Asn	...	...	...	...	...	...
CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val	...	...
CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val	...	...
BMP3	...	Glu	Asn	Lys	...	...	Val	...	...
GDF-1	...	Asn	...	Asp	...	...	Val	...	Arg

60A	Leu	Asn	Asp	Glu	...	...	Asn	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Asn	...	...	...	...	...	...
					85				

hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP-1	...	...	...	...	...	...	...	...
hOP-2	...	His	...	...	...	...	...	Lys
mOP-2	...	His	...	...	...	...	...	Lys
DPP	Asn	...	Gln	Glu	...	Thr	...	Val
Vgl	His	...	Glu	...	...	Ala	...	Asp
Vgr-1	...	...	...	...	...	...	...	...
CBMP-2A	Asn	...	Gln	Asp	...	...	...	Glu
CBMP-2B	Asn	...	Gln	Glu	...	...	...	Glu
BMP3	Val	...	Pro	...	...	Thr	...	Glu
GDF-1	Gln	...	Glu	Asp	...	...	...	Asp
60A	...	...	...	...	...	Ile	...	Lys
BMP5	...	...	...	...	...	...	...	...
BMP6	...	...	...	Trp	...	...	...	...
	90					95		

hOP-1	Ala	Cys	Gly	Cys	His
mOP-1	...	...	...	...	...
hOP-2	...	...	...	...	...
mOP-2	...	...	...	...	...
DPP	Gly	...	...	...	Arg
Vgl	Glu	...	...	...	Arg
Vgr-1	...	...	...	...	...
CBMP-2A	Gly	...	...	...	Arg
CBMP-2B	Gly	...	...	...	Arg

BMP3	Ser	...	Ala	...	Arg
GDF-1	Glu	...	...	...	Arg
60A	Ser	...	...	...	...
BMP5	Ser	...	...	...	...
BMP6	...	...	...	...	...
			100		

**Between residues 56 and 57 of BMP3 is a Val residue; between residues 43 and 44 of GDF-1 lies the amino acid sequence Gly-Gly-Pro-Pro.

As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the generic sequences while retaining the morphogenic activity. For example, while the GDF-1 protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP1 sequence, where "homology" or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in still another

preferred aspect, the invention includes morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

II. Formulations and Methods for Administering Therapeutic Agents

The morphogens may be provided to an individual by any suitable means, preferably directly (e.g., locally, as by injection to a nerve tissue locus) or systemically (e.g., parenterally or orally). Where the morphogen is to be provided parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (9.85% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol

containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. For example, association of the mature dimer with the pro domain of the morphogen increases solubility of the protein significantly. In fact, the endogenous protein is thought to be transported in this form. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, polybutyrate, tricalcium phosphate, lactide and lactide/glycolide copolymers, may be useful excipients to control the release of the morphogen in vivo. Other potentially useful parenteral delivery systems for these morphogens

include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or cutric acid for vaginal administration.

Alternatively, the morphogens described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the morphogens described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat.No. 4,968,590.) In addition, at least one morphogen, OP-1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from mammary gland extract is morphogenically active. Specifically, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone assay, such as is disclosed in U.S. Pat.No. 4,968,590. Moreover, the morphogen also is detected in the bloodstream (see Example 9, below). Finally, soluble form morphogen, e.g., mature morphogen associated with the pro domain, is capable of maintaining neural pathways in a mammal (See Examples 4

and 6 below). These findings indicate that oral and parenteral administration are viable means for administering morphogens to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

The compounds provided herein also may be associated with molecules capable of targeting the morphogen or morphogen-stimulating agent to nerve tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on nerve tissue cells, including neuronal or glial cells, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As described above, the morphogens provided herein share significant sequence homology in the C-terminal active domains. By contrast, the sequences typically diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain is thought to be morphogen-specific. As described above, it is also known that the various morphogens identified to date are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the

body, selected morphogens typically act on a given tissue. Accordingly, part or all of the pro domains which have been identified associated with the active form of the morphogen in solution, may serve as targeting molecules for the morphogens described herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Accordingly, another useful targeting molecule for targeting morphogen to nerve tissue is part or all of a morphogen pro domain, particularly part or all of the pro domains of OP-1 or GDF-1, both of which proteins are found naturally associated with nerve tissue.

Finally, the morphogens or morphogen-stimulating agents provided herein may be administered alone or in combination with other molecules known to be beneficial in maintaining neural pathways, including nerve growth factors and anti-inflammatory agents.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and carriers. As noted above, such compositions may be prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols.

The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations for a time

sufficient to eliminate or reduce the patient's pathological condition, to provide therapy for the neurological diseases and disorders described above, and amounts effective to enhance neural cell survival an/or to protect neurons and neural pathways in anticipation of injury to nerve tissue.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such variables as the type and extent of progression of the neurological disease, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound excipients, and its route of administration. In general terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1 μg/kg to 100 mg/kg of body weight per day. Optimally, the morphogen dosage given in all cases is between 2-20 μg of protein per kilogram weight of the patient per day. No obvious OP-1 induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 μg) is

administered daily to normal growing rats for 21 consecutive days. Moreover, 10 μg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalties.

Since the ability of proteins and protein fragments to penetrate the blood-brain barrier may be related to their size, lipophilicity or their net ionic charge, suitable modifications of the morphogens may be formulated (e.g., by substituting pentafluorophenylalanine for phenylalanine, or by conjugation to a cationized protein such as albumin) to increase their transportability across the barrier, using standard methodologies known in the art. See, for example, Kastin et al., <u>Pharmac. Biochem. Behav.</u> (1979) <u>11</u>:713-716; Rapoport et al., <u>Science</u> (1980) <u>207</u>:84-86; Pardridge et al., (1987) <u>Biochem. Biophys. Res. Commun.</u> <u>146</u>:307-313; Riekkinen et al.,(1987) <u>Peptides</u> <u>8</u>:261-265. The efficacy of these functional analogs may be assessed for example, by evaluating the ability of these compounds to induce redifferentiation of transformed cells, or enhance survival of neurons at risk of dying, as described in the Examples provided herein.

In administering morphogens systemically in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

Where injury to neurons of a neural pathway is induced deliberately as part of, for example, a surgical procedure, the morphogen preferably is provided just prior to, or concomitant with induction of the trauma. Preferably, the morphogen is administered prophylactically in a surgical setting. Optimally, the morphogen dosage given in all cases is between 2-20 μg of protein per kilogram weight of the patient.

Alternatively, an effective amount of an agent capable of stimulating endogenous morphogen levels may be administered by any of the routes described above. For example, an agent capable of stimulating morphogen production and/or secretion from nerve tissue cells may be provided to a mammal, e.g., by direct administration of the morphogen to glial cells associated with the nerve tissue to be treated. A method for identifying and testing agents capable of modulating the levels of endogenous morphogens in a given tissue is described generally herein in Example 13, and in detail in copending USSN 752,859, filed August 30, 1991, the disclosure of which is incorporated herein by reference. Briefly, candidate compounds can be identified and tested by incubating the compound <u>in vitro</u> with a test tissue or cells thereof, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue. Here, suitable tissue or cultured cells of a tissue preferably would comprise neurons and/or glial cells. For example, suitable tissue for testing may include cultured cells isolated from the substantia nigra, adendema glial cells, and the like.

A currently preferred detection means for evaluating the level of the morphogen in culture upon exposure to the candidate compound comprises an immunoassay utilizing an antibody or other suitable binding protein capable of reacting specifically with a morphogen and being detected as part of a complex with the morphogen. Immunoassays may be performed using standard techniques known in the art and antibodies raised against a morphogen and specific for that morphogen. As described herein, morphogens may be isolated from natural-sourced material or they may be recombinantly produced. Agents capable of stimulating endogenous morphogens then may formulated into pharmaceutical preparations and administered as described herein.

Where the morphogen is to be provided to a site to stimulate axon regeneration, the morphogen preferably is provided to the site in association with a biocompatible, preferably bioresorbable carrier suitable for maintaining a protein at a site _in vivo_, and through which a neural process may regenerate. A currently preferred carrier also comprises sufficient structure to assist direction of axonal growth. Currently preferred carriers include structural molecules such as collagen, hyaluronic acid or laminin, and/or synthetic polymers or copolymers of, for example, polylactic acid, polyglycolic acid or polybutyric acid. Currently most preferred are carriers comprising tissue extracellular matrix. These may be obtained commercially. In addition, a brain tissue-derived extracellular matrix may be prepared as described in USSN 752,264, incorporated hereinabove by reference, and/or by other means known in the art.

The currently preferred means for repairing breaks in neural pathways, particularly pathways of the peripheral nervous system, include providing the morphogen to the site as part of a device that includes a biocompatible membrane or casing of a dimension sufficient to span the break and having openings adapted to receive severed nerve ends. The morphogen is disposed within the casing, preferably dispersed throughout a suitable carrier, and is accessible to the severed nerve ends. Alternatively, the morphogen may be adsorbed onto the interior surface of the casing, or otherwise associated therewith. In addition, currently preferred casings have an impermeable exterior surface. The casing acts as a nerve guidance channel, aiding in directing axonal growth. In addition, the casing also protects the damaged nerve from immunologically-related agents which may assist in scar tissue formation. Suitable channel or casing materials include silicone or bioresorbable materials such as collagen, hyaluronic acid, laminin, polylactic acid, polyglycolic acid, polybutyric acid and the like. Additionally, although the nerve guidance channels described herein generally are tubular in shape, it should be evident to those skilled in the art that various alternative shapes may be employed. The lumen of the guidance channels may, for example, be oval or even square in cross section. Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may be secured to the nerve guidance channels by means of sutures, biocompatible adhesives such as fibrin glue, or other means known in the art.

III. Examples

Example 1. Identification of Morphogen-Expressing Tissue

Determining the tissue distribution of morphogens may be used to identify different morphogens expressed in a given tissue, as well as to identify new, related morphogens. Tissue distribution also may be used to identify useful morphogen-producing tissue for use in screening and identifying candidate morphogen-stimulating agents. The morphogens (or their mRNA transcripts) readily are identified in different tissues using standard methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or immunofluorescent techniques, and antibodies specific to the morphogen or morphogens of interest. Similarly, the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens described herein share such high sequence homology in their active, C-terminal domains, the tissue distribution of a specific morphogen transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein. Another useful sequence is the 3′ non-coding region flanking and immediately following the stop codon. These portions of the sequence vary substantially among

the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence). Similarly, particularly useful mOP-1-specific probe sequences are the BstX1-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain; and the Ear1-Pst1 fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP-1 (Seq. ID No. 16) or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart, brain, thymus and stomach) by a standard methodology such as by the method of Chomczyaski et al. ((1987) Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 μg) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the

transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 mW/cm^2). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% formamide, 5 x Denhardts, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of various morphogens, including Vgr-1, OP-1, BMP2, BMP3, BMP4, BMP5, GDF-1, and OP-2 in developing and adult tissue are disclosed in co-pending USSN 752,764, and in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commn. 179:116-123, and Ozkaynak, et al. (1992) (JBC, in press), the disclosures of which are incorporated herein by reference. Using the general probing methodology described herein, northern blot hybridizations using probes specific for these morphogens to probe brain, spleen, lung, heart, liver and kidney tissue indicate that kidney-related tissue appears to be the primary expression source for OP-1, with brain, heart and lung tissues being secondary sources. Lung tissue appears to be the primary tissue expression source for Vgr-1, BMP5, BMP4 and BMP3. Lower levels of Vgr-1 also are seen in kidney and heart tissue, while the liver appears to be a secondary expression source for BMP5, and the spleen appears to be a secondary expression source for BMP4. GDF-1 appears to be expressed primarily in brain tissue. To date, OP-2 appears to be expressed primarily in early

embryonic tissue. Specifically, northern blots of murine embryos and 6-day post-natal animals shows abundant OP2 expression in 8-day embryos. Expression is reduced significantly in 17-day embryos and is not detected in post-natal animals.

Example 2. Morphogen Localization in the Nervous System

Morphogens have been identified in developing and adult rat brain and spinal cord tissue, as determined both by northern blot hybridization of morphogen-specific probes to mRNA extracts from developing and adult nerve tissue (see Example 1, above) and by immunolocalization studies. For example, northern blot analysis of developing rat tissue has identified significant OP-1 mRNA transcript expression in the CNS (USSN 752,764, and Ozkaynak et al. (1991) Biochem. Biophys. Res. Comm., 179:11623 and Ozkaynak, et al. (1992) JBC, in press). GDF-1 mRNA appears to be expressed primarily in developing and adult nerve tissue, specifically in the brain, including the cerebellum and brain stem, spinal cord and peripheral nerves (Lee, S. (1991) PNAS 88: 4250-4254). BMP2B (also referred in the art as BMP4) and Vgr-1 transcripts also have been reported to be expressed in nerve tissue (Jones et al. (1991) Development 111:531-542), although the nerve tissue does not appear to be the primary expression tissue for these genes (Ozkaynak, et al., (1992) JBC in press). Specifically, CBMP2 transcripts are reported in the region of the diencephalon associated with pituitary development, and Vgr-1 transcripts are reported in the anteroposterior axis of the CNS, including in the roof plate of the developing neural tube, as well as in the cells

immediately adjacent the floor plate of the developing neural tube. In older rats, Vgr-1 transcripts are reported in developing hippocampus tissue. In addition, the genes encoding OP-1 and BMP2 originally were identified by probing human hippocampus cDNA libraries.

Immunolocalization studies, performed using standard methodologies known in the art and disclosed in USSN 752,764, filed August 30, 1991, the disclosure of which is incorporated herein, localized OP-1 expression to particular areas of developing and adult rat brain and spinal cord tissue. Specifically, OP-1 protein expression was assessed in adult (2-3 months old) and five or six-day old mouse embryonic nerve tissue, using standard morphogen-specific antisera, specifically, rabbit anti-OP1 antisera, made using standard antibody protocols known in the art and preferably purified on an OP-1 affinity column. The antibody itself was labelled using standard fluorescent labelling techniques, or a labelled anti-rabbit IgG molecule was used to visualize bound OP-1 antibody.

As can be seen in FIG 1A and 1B, immunofluorescence staining demonstrates the presence of OP-1 in adult rat central nervous system (CNS.) Similar and extensive staining is seen in both the brain (1A) and spinal cord (1B). OP-1 appears to be localized predominantly to the extracellular matrix of the grey matter (neuronal cell bodies), distinctly present in all areas except the cell bodies themselves. In white matter, formed mainly of myelinated nerve fibers, staining appears to be confined to astrocytes (glial cells). A similar staining pattern also was seen in newborn rat (10 day old) brain sections.

In addition, OP-1 has been specifically localized in the substantia nigra, which is composed primarily of striatal basal ganglia, a system of accessory motor neurons that function is association with the cerebral cortex and corticospinal system. Dysfunctions in this subpopulation or system of neurons are associated with a number of neuropathies, including Huntington's chorea and Parkinson's disease.

OP1 also has been localized at adendema glial cells, known to secrete factors into the cerebrospinal fluid, and which occur around the intraventricular valve, coroid fissure, and central canal of the brain in both developing and adult rat.

Finally, morphogen inhibition in developing embryos inhibits nerve tissue development. Specifically, 9-day mouse embryo cells, cultured in vitro under standard culturing conditions, were incubated in the presence and absence of an OP-1-specific monoclonal antibody prepared using recombinantly produced, purified mature OP-1 and the immunogen. The antibody was prepared using standard antibody production means well known in the art and as described generally in Example 13. After two days, the effect of the antibody on the developing embryo was evaluated by histology. As determined by histological examination, the OP-1-specific antibody specifically inhibits eye lobe formation in the developing embryo. In particular, the diencephalon outgrowth does not develop. In addition, the heart is malformed and enlarged. Moreover, in separate immunolocalization studies on embryo sections with labelled OP-1 specific antibody, the OP-1-specific antibody localizes to neural epithelia.

The endogenous morphogens which act on neuronal cells may be expressed and secreted by nerve tissue cells, e.g., by neurons and/or glial cells associated with the neurons, and/or they may be transported to the neurons by the cerebrospinal fluid and/or bloodstream. Recently, OP-1 has been identified in the human blood (See Example 9, below). In addition, transplanted Schwann cells recently have been shown to stimulate nerve fiber formation in rat spinal cord, including inducing vascularization and myelin sheath formation around at least some of the new neuronal processes (Bunge (1991) Exp. Neurology 114:254-257.) The regenerative property of these cells may be mediated by the secretion of a morphogen by the Schwann cells.

Example 3. Morphogen Enhancement of Neuronal Cell Survival

The morphogens described herein enhance cell survival, particularly of neuronal cells at risk of dying. For example, fully differentiated neurons are non-mitotic and die *in vitro* when cultured under standard mammalian cell culture conditions, using a chemically defined or low serum medium known in the art, (see, for example, Charness (1986) J. Biol. Chem. 26:3164-3169 and Freese et al. (1990) Brain Res. 521:254-264.) However, if a primary culture of non-mitotic neuronal cells is treated with a morphogen, the survival of these cells is enhanced significantly. For example, a primary culture of striatal basal ganglia isolated from the substantia nigra of adult rat brain was prepared using standard procedures, e.g., by dissociation by trituration with pasteur pipette of substania nigra tissue, using standard tissue culturing protocols, and grown in a low serum medium, e.g.,

containing 50% DMEM (Dulbecco's modified Eagle's medium), 50% F-12 medium, heat inactivated horse serum supplemented with penicillin/streptomycin and 4 g/l glucose. Under standard culture conditions, these cells are undergoing significant cell death by three weeks when cultured in a serum-free medium. Cell death is evidenced morphologically by the inability of cells to remain adherent and by changes in their ultrastructural characteristics, e.g., by chromatin clumping and organelle disintegration.

In this example, the cultured basal ganglia were were treated with chemically defined medium conditioned with 0.1-100 ng/ml OP-1. Fresh, morphogen-conditioned medium was provided to the cells every 3-4 days. Cell survival was enhanced significantly and was dose dependent upon the level of OP-1 added: cell death decreased significantly as the concentration of OP-1 was increased in cell cultures. Specifically, cells remained adherent and continued to maintain the morphology of viable differentiated neurons. In the absence of morphogen treatment, the majority of the cultured cells dissociated and underwent cell necrosis.

Dysfunctions in the basal ganglia of the sustantia nigra are associated with Huntington's chorea and parkinsonism <u>in vivo</u>. The ability of the morphogens defined herein to enhance neuron survival indicates that these morphogens will be useful as part of a therapy to enhance survival of neuronal cells at risk of dying <u>in vivo</u> due, for example, to a neuropathy or chemical or mechanical trauma. It is particularly anticipated that these morphogens will provide a useful therapeutic agent to treat neuropathies which affect the striatal basal ganglia, including Huntington's

chorea and Parkinson's disease. For clinical applications, the morphogen may be administered or, alternatively, a morphogen-stimulating agent may be administered.

Example 4. <u>Morphogen-Induced Redifferentiation of Transformed Cells</u>

The morphogens described herein also induce redifferentiation of transformed cells to a morphology characteristic of untransformed cells. In particular, the morphogens are capable of inducing redifferentiation of transformed cells of neuronal origin to a morphology characteristic of untransformed neurons. The example provided below details morphogen induced redifferentiation of a transformed human cell line of neuronal origin, NG105-115. Morphogen-induced redifferentiation of transformed cells also has been shown in mouse neuroblastoma cells (N1E-115) and in human embryo carcimona cells (see copending USSN 752,764, incorporated herein by reference.)

NG108-15 is a transformed hybrid cell line produced by fusing neuroblastoma x glioma cells (obtained from America Type Tissue Culture, Rockville, MD), and exhibiting a morphology characteristic of transformed embryonic neurons, e.g., having a fibroblastic morphology. Specifically, the cells have polygonal cell bodies, short, spike-like processes and make few contacts with neighboring cells (see FIG. 1A). Incubation of NG108-15 cells, cultured in a chemically defined, serum-free medium, with 0.1 to 300 ng/ml of OP-1 for four hours induces an orderly, dose-dependent change in cell morphology.

In the experiment NG108-15 cells were subcultured on poly-L-lysine coated 6-well plates. Each well contained 40-50,000 cells in 2.5 ml of chemically defined medium. On the third day 2.5 μl of OP-1 in 60% ethanol containing 0.025% trifluoroacetic was added to each well. OP-1 concentrations of 0-300 ng/ml were tested. Typically, the media was changed daily with new aliquots of OP-1, although morphogenesis can be induced by a single four hour incubation with OP-1. In addition, OP-1 concentrations of 10 ng/ml were sufficient to induce redifferentiation. After one day, hOP-1-treated cells undergo a significant change in their cellular ultrastructure, including rounding of the soma, increase in phase brightness and extension of the short neurite processes. After two days, cells treated with OP-1 begin to form epithelioid sheets, which provide the basis for the growth of mutilayered aggregates at three day's post-treatment. By four days, the great majority of OP-1-treated cells are associated in tightly-packed, mutilayered aggregates (Fig. 1B). Fig. 2 plots the mean number of multi-layered aggregates or cell clumps identified in twenty randomly selected fields from six independent experiments, as a function of morphogen concentration. Forty ng/ml of OP-1 is sufficient for half maximum induction of cell aggregation.

The morphogen-induced redifferentiation occurred without any associated changes in DNA synthesis, cell division, or cell viability, making it unlikely that the morphologic changes were secondary to cell differentiation or a toxic effect of hOP-1. Moreover, the OP-1-induced morphogenesis does not inhibit cell division, as determined by ^{3}H-thymidine uptake, unlike other molecules which have been shown to stimulate

differentiation of transformed cells, such as butyrate, DMSO, retanoic acid or Forskolin. The data indicate that OP-1 can maintain cell stability and viability after inducing redifferentiation. In addition, the effects are morphogen specific, and redifferentiation is not induced when NG108-15 cells are incubated with 0.1-40 ng/ml TGF-β.

The experiments also have been performed with highly purified soluble morphogen (e.g., mature OP1 associated with its pro domain) which also specifically induced redifferentiation of NG108-15 cells.

The morphogens described herein accordingly provide useful therapeutic agents for the treatment of neoplasias and neoplastic lesions of the nervous system, particularly in the treatment of neuroblastomas, including retinoblastomas, and gliomas. The morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

Example 5. Nerve Tissue Protection from Chemical Trauma

The ability of the morphogens described herein to enhance survival of neuronal cells and to induce cell aggregation and cell-cell adhesion in redifferentiated cells, indicates that the morphogens will be useful as therapeutic agents to maintain neural pathways by protecting the cells defining the pathway from the damage caused by chemical trauma. In particular, the morphogens can protect neurons, including developing neurons, from the effects of toxins known to inhibit

the proliferation and migration of neurons and to interfere with cell-cell adhesion. Examples of such toxins include ethanol, one or more of the toxins present in cigarette smoke, and a variety of opiates. The toxic effects of ethanol on developing neurons induces the neurological damage manifested in fetal alcohol syndrome. The morphogens also may protect neurons from the cytoxic effects associated with excitatory amino acids such as glutamate.

For example, ethanol inhibits the cell-cell adhesion effects induced in morphogen-treated NG108-15 cells when provided to these cells at a concentration of 25-50 mM. Half maximal inhibition can be achieved with 5-10 mM ethanol, the concentration of blood alcohol in an adult following ingestion of a single alcoholic beverage. Ethanol likely interferes with the homophilic binding of CAMs between cells, rather than their induction, as morphogen-induced N-CAM levels are unaffected by ethanol. Moreover, the inhibitory effect is inversely proportional to morphogen concentration. Accordingly, it is envisioned that administration of a morphogen or morphogen-stimulating agent to neurons, particularly developing neurons, at risk of damage from exposure to toxins such as ethanol, may protect these cells from nerve tissue damage by overcoming the toxin's inhibitory effects. The morphogens described herein also are useful in therapies to treat damaged neural pathways resulting from a neuropathy induced by exposure to these toxins.

Example 6. <u>Morphogen-Induced CAM Expression</u>

The morphogens described herein induce CAM expression, particularly N-CAM expression, as part of their induction of morphogenesis. CAMs are morphoregulatory molecules identified in all tissues as an essential step in tissue development. N-CAMs, which comprise at least 3 isoforms (N-CAM-180, N-CAM-140 and N-CAM-120, where "180", "140" and "120" indicate the apparent molecular weights of the isoforms as measured by polyacrylamide gel electrophoresis) are expressed at least transiently in developing tissues, and permanently in nerve tissue. Both the N-CAM-180 and N-CAM-140 isoforms are expressed in both developing and adult tissue. The N-CAM-120 isoform is found only in adult tissue. Another neural CAM is L1.

N-CAMs are implicated in appropriate neural development, including appropriate nuerulation, neuronal migration, fasciculation, and synaptogenesis. Inhibition of N-CAM production, as by complexing the molecule with an N-CAM-specific antibody, inhibits retina organization, including retinal axon migration, and axon regeneration in the peripheral nervous system, as well as axon synapsis with target muscle cells. In addition, significant evidence indicates that physical or chemical trauma to neurons, oncogenic transformation and some genetic neurological disorders are accompanied by changes in CAM expression, which alter the adhesive or migratory behavior of these cells. Specifically, increased N-CAM levels are reported in Huntington's disease striatum (e.g., striatal basal ganglia), and decreased adhesion is noted in Alzheimer's disease.

The morphogens described herein can stimulate CAM production, particularly L1 and N-CAM production, including all three isoforms of the N-CAM molecule. For example, N-CAM expression is stimulated significantly in morphogen-treated NG108-15 cells. Untreated NG108-15 cells exhibit a fibroblastic, or minimally differentiated morphology and express only the 180 and 140 isoforms of N-CAM normally associated with a developing cell. Following morphogen treatment these cells exhibit a morphology characteristic of adult neurons and express enhanced levels of all three N-CAM isoforms. Using a similar protocol as described in the example below, morphogen treatment of NG108-15 cells also induced L1 expression.

In this example NG108-15 cells were cultured for 4 days in the presence of increasing concentrations of OP-1 and standard Western blots performed on whole cells extracts. N-CAM isoforms were detected with an antibody which crossreacts with all three isoforms, mAb H28.123, obtained from Sigma Chemical Co., St. Louis, the different isoforms being distinguishable by their different mobilities on an electrophoresis gel. Control NG108-15 cells (untreated) express both the 140 kDa and the 180 kDa isoforms, but not the 120 kDa, as determined by western blot analyses using up to 100 μg of protein. Treatment of NG108-15 cells with OP-1 resulted in a dose-dependent increase in the expression of the 180 kDa and 140 kDa isoforms, as well as the induction of the 120 kDa isoform. See Fig. 2A and 2B. Fig. 2B is a Western blot of OP1-treated NG108-15 cell extracts, probed with mAb H28.123, showing the induction of all three isoforms. Fig. 2A is a dose response curve of N-CAM-180 and N-CAM-140 induction as a function of morphogen concentration. N-

CAM-120 is not shown in the graph as it could not be quantitated in control cells. However, as is clearly evident from the Western blot in Fig. 2A, N-CAM-120 is induced in response to morphogen treatment. The differential induction of N-CAM 180 and 140 isoforms seen may be because constitutive expression of the 140 isoform is close to maximum.

The increase in N-CAM expression corresponded in a dose-dependent manner with the morphogen induction of multicellular aggregates. Compare Fig. 2A and Fig 3. Fig. 3 graphs the mean number of multilayered aggregates (clumps) counted per 20 randomly selected fields in 6 independent experiments, versus the concentration of morphogen. The induction of the 120 isoform also indicates that morphogen-induced redifferentiation of transformed cells stimulates not only redifferentiation of these cells from a transformed phenotype, but also differentiation to a phenotype corresponding to a developed cell. Standard immunolocalization studies performed with the mAb H28.123 on morphogen-treated cells show N-CAM cluster formation associated with the periphery and processes of treated cells and no reactivity with untreated cells. Moreover, morphogen treatment does not appear to inhibit cell division as determined by cell counting or ^{3}H-thymidine uptake. Finally, known chemical differentiating agents, such as Forskolin and dimethylsulfoxide do not induce N-CAM production.

In addition, the cell aggregation effects of OP-1 on NG108-15 cells can be inhibited with anti-N-CAM antibodies or antisense N-CAM oligonucleotides. Antisense oligonucleotides can be made synthetically on a nucleotide synthesizer, using standard means known in

the art. Preferably, phosphorothioate oligonucleotides ("S-oligos") are prepared, to enhance transport of the nucleotides across cell membranes. Concentrations of both N-CAM antibodies and N-CAM antisense oliognucleotides sufficient to inhibit N-CAM induction also inhibited formation of multilayered cell aggregates. Specifically, incubation of morphogen-treated NG108-115 cells with 0.3-3 μM N-CAM antisense S-oligos, 5-500 μM unmodified N-CAM antisense oligos, or 10 μg/ml mAb H28.123 significantly inhibits cell aggregation. It is likely that morphogen treatment also stimulates other CAMs, as inhibition is not complete.

The experiments also have been performed with soluble morphogen (e.g., mature OP-1 associated with its pro domain) which also specifically induced CAM expression.

The morphogens described herein are useful as therapeutic agents to treat neurological disorders associated with altered CAM levels, particularly N-CAM levels, such as Huntington's chorea and Alzheimers' disease, and the like. In clinical applications, the morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

The efficacy of the morphogens described herein to affect N-CAM expression may be assessed <u>in vitro</u> using a suitable cell line and the methods described herein. In addition to a transformed cell line, N-CAM expression can be assayed in a primary cell culture of neural or glial cells, following the procedures described herein. The efficacy of morphogen treatment

on N-CAM expression in vivo may be evaluated by tissue biopsy as described in Example 9, below, and detecting N-CAM molecules with an N-CAM-specific antibody, such as mAb H28.123, or using the animal model described in Example 11.

Alternatively, the level of N-CAM proteins or protein fragments present in cerebrospinal fluid or serum also may be detected to evaluate the effect of morphogen treatment. N-CAM molecules are known to slough off cell surfaces and have been detected in both serum and cerebrospinal fluid. In addition, altered levels of the soluble form of N-CAM are associated with normal pressure hydrocephalus and type II schizophrenia. N-CAM fluid levels may be detected following the procedure described in Example 9 and using an N-CAM specific antibody, such as mAb H28.123.

Example 7. Morphogen-Induced Nerve Gap Repair (PNS)

The morphogens described herein also stimulate peripheral nervous system axonal growth over extended distances allowing repair and regeneration of damaged neural pathways. While neurons of the peripheral nervous system can sprout new processes following injury, without guidance these sproutings typically fail to connect appropriately and die. Where the break is extensive, e.g., greater than 5 or 10 mm, regeneration is poor or nonexistent.

In this example morphogen stimulation of nerve regeneration was assessed using the rat sciatic nerve model. The rat sciatic nerve can regenerate spontaneously across a 5 mm gap, and occasionally

across a 10 mm gap, provided that the severed ends are inserted in a saline-filled nerve guidance channel. In this experiment, nerve regeneration across a 12mm gap was tested.

Adult female Sprague-Dawley rats (Charles River Laboratories, Inc.) weighing 230-250 g were anesthetized with intraperitoneal injections of sodium pentobarbital 35 mg/kg body weight). A skin incision was made parallel and just posterior to the femur. The avascular intermuscular plane between vastus lateralis and hamstring muscles were entered and followed to the loose fibroareolar tissue surrounding the sciatic nerve. The loose tissue was divided longitudinally thereby freeing the sciatic nerve over its full extent without devascularizing any portion. Under a surgical microscope the sciatic nerves were transected with microscissors at mid-thigh and grafted with an OP-1 gel graft that separated the nerve stumps by 12 mm. The graft region was encased in a silicone tube 20 mm in length with a 1.5 mm inner diameter,the interior of which was filled a morphogen solution. Specifically, The central 12 mm of the tube consisted of an OP-1 gel prepared by mixing 1 to 5 μg of substantially pure CHO-produced recombinant OP-1 with approximately 100 μl of MATRIGEL^{TM} (from Collaborative Research, Inc., Bedford, MA), an extracellular matrix extract derived from mouse sarcoma tissue, and containing solubilized tissue basement membrane, including laminin, type IV collagen, heparin sulfate, proteoglycan and entactin, in phosphate-buffered saline. The OP-1-filled tube was implanted directly into the defect site, allowing 4 mm

on each end to insert the nerve stumps. Each stump was abutted against the OP-1 gel and was secured in the silicone tube by three stitches of commercially available surgical 10-0 nylon through the epineurium, the fascicle protective sheath.

In addition to OP-1 gel grafts, empty silicone tubes, silicone tubes filled with gel only and "reverse" autografts, wherein 12 mm transected segments of the animal's sciatic nerve were rotated 180° prior to suturing, were grafted as controls. All experiments were performed in quadruplicate. All wounds were closed by wound clips that were removed after 10 days. All rats were grafted on both legs. At 3 weeks the animals were sacrificed, and the grafted segments removed and frozen on dry ice immediately. Frozen sections then were cut throughout the graft site, and examined for axonal regeneration by immunofluorescent staining using anti-neurofilament antibodies labeled with flurocein (obtained from Sigma Chemical Co., St. Louis).

Regeneration of the sciatic nerve occurred across the entire 12 mm distance in all graft sites wherein the gap was filled with the OP-1 gel. By contrast, empty silicone tubes and reverse autografts did not show nerve regeneration, and only one graft site containing the gel alone showed axon regeneration.

Example 8. Morphogen-Induced Nerve Gap Repair (CNS)

Following axonal damage *in vivo* the CNS neurons are unable to resprout processes. Accordingly, trauma to CNS nerve tissue, including the spinal cord, optic nerve and retina, severely damages or destroys the neural pathways defined by these cells. Peripheral nerve grafts have been used in an effort to bypass CNS axonal damage. Successful autologous graft repair to date apparently requires that the graft site occur near the CNS neuronal cell body, and a primary result of CNS axotomy is neuronal cell death. The efficacy of morphogens described herein on CNS nerve repair, may be evaluated using a rat crushed optic nerve model such as the one described by Bignami et al., (1979) Exp. Eye Res. 28: 63-69, the disclosure of which is incorporated herein by reference. Briefly, and as described therein, laboratory rats (e.g., from Charles River Laboratories, Wilmington, MA) are anesthesized using standard surgical procedures, and the optic nerve crushed by pulling the eye gently out of the orbit, inserting a watchmaker forceps behind the eyeball and squeezing the optic nerve with the forceps for 15 seconds, followed by a 30 second interval and second 15 second squeeze. Rats are sacrificed at different time intervals, e.g., at 48 hours, and at 3, 4, 11, 15 and 18 days after operation. The effect of morphogen on optic nerve repair can be assessed by performing the experiment in duplicate and providing morphogen or PBS (e.g., 25 μl solution, and 25 μg morphogen) to the optic nerve, e.g., just prior to the operation, concommitant with the operation, or at specific times after the operation.

In the absence of therapy, the surgery induces glial scarring of the crushed nerve, as determined by immunofluoresence staining for glial fibrillary acidic protein (GFA), a marker protein for glial scarring, and by histology. Indirect immunofluoresence on air-dried cryostat sections as described in Bignami et al. (1974) J. Comp. Neur. 153: 27-38, using commercially available antibodies to GFA (e.g., Sigma Chemical Co., St. Louis). Reduced levels of GFA are anticipated in animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation and to stimulate optic nerve regeneration.

Example 9. Nerve Tissue Diagnostics

Morphogen localization in nerve tissue can be used as part of a method for diagnosing a neurological disorder or neuropathy. The method may be particularly advantageous for diagnosing neuropathies of brain tissue. Specifically, a biopsy of brain tissue is performed on a patient at risk, using standard procedures known in the medical art. Morphogen expression associated with the biopsied tissue then is assessed using standard methodologies, as by immunolocalization, using standard immunofluorescence techniques in concert with morphogen-specific antisera or monoclonal antibodies. Specifically, the biopsied tissue is thin sectioned using standard methodologies known in the art, and fluorescently labelled (or otherwise detectable) antibodies incubated with the tissue under conditions sufficient to allow specific antigen-antibody complex formation. The presence and quantity of complex formed then is detected and compared with a predetermined standard or reference

value. Detection of altered levels of morphogen present in the tissue then may be used as an indicator of tissue dysfunction. Alternatively, fluctuation in morphogen levels may be assessed by monitoring morphogen transcription levels, either by standard northern blot analysis or in situ hybridization, using a labelled probe capable of hybridizing specifically to morphogen RNA and standard RNA hybridization protocols well described in the art.

Fluctuations in morphogen levels present in the cerebrospinal fluid or bloodstream also may be used to evaluate nerve tissue viability. For example, morphogens are detected associated with adendema cells which are known to secrete factors into the cerebrospinal fluid, and are localized generally associated with glial cells, and in the extracellular matrix, but not with neuronal cell bodies. Accordingly, the cerebrospinal fluid may be a natural means of morphogen transport. Alternatively, morphogens may be released from dying cells into cerebrospinal fluid. In addition, OP-1 recently has been identified in human blood, which also may be a means of morphogen transport, and/or a repository for the contents of dying cells.

Spinal fluid may be obtained from an individual by a standard lumbar puncture, using standard methodologies known in the medical art. Similarly, serum samples may be obtained by standard venipuncture and serum prepared by centrifugation at 3,000 RPM for ten minutes. The presence of morphogen in the serum or cerebral spinal fluid then may be assessed by standard Western blot (immunoblot), ELISA or RIA procedures. Briefly, for example, with the ELISA, samples may be

diluted in an appropriate buffer, such as phosphate-buffered saline, and 50 μl aliquots allowed to absorb to flat bottomed wells in microtitre plates pre-coated with morphogen-specific antibody, and allowed to incubate for 18 hours at 4°C. Plates then may be washed with a standard buffer and incubated with 50 μl aliquots of a second morphogen-specific antibody conjugated with a detecting agent, e.g., biotin, in an appropriate buffer, for 90 minutes at room temperature. Morphogen-antibody complexes then may be detected using standard procedures.

Alternatively, a morphogen-specific affinity column may be created using, for example, morphogen-specific antibodies adsorbed to a column matrix, and passing the fluid sample through the matrix to selectively extract the morphogen of interest. The morphogen then is eluted. A suitable elution buffer may be determined empirically by determining appropriate binding and elution conditions first with a control (e.g., purified, recombinantly-produced morphogen.) Fractions then are tested for the presence of the morphogen by standard immunoblot, and confirmed by N-terminal sequencing. Morphogen concentrations in serum or other fluid samples then may be determined using standard portein quantification techniques, including by spectrophotometric absorbance or by quantitation by ELISA or RIA antibody assays. Using this procedure, OP-1 has been identified in serum.

OP-1 was detected in human serum using the following assay. A monoclonal antibody raised against mammalian, recombinantly produced OP-1 using standard immunology techniques well described in the art and described generally in Example 13, was immobilized by

passing the antibody over an activated agarose gel (e.g., Affi-Gel™, from Bio-Rad Laboratories, Richmond, CA, prepared following manufacturer's instructions), and used to purify OP-1 from serum. Human serum then was passed over the column and eluted with 3M K-thiocyanate. K-thiocyanante fractions then were dialyzed in 6M urea, 20mM PO_4, pH 7.0, applied to a C8 HPLC column, and eluted with a 20 minute, 25-50% acetonitrile/0.1% TFA gradient. Mature, recombinantly produced OP-1 homodimers elute between 20-22 minutes. Fractions then were collected and tested for the presence of OP-1 by standard immunoblot. Fig. 4 is an immunoblot showing OP-1 in human sera under reducing and oxidized conditions. In the figure, lanes 1 and 4 are OP-1 standards, run under oxidized (lane 1) and reduced (lane 4) conditions. Lane 5 shows molecular weight markers at 17, 27 and 39 kDa. Lanes 2 and 3 are human sera OP-1, run under oxidized (lane 2) and reduced (lane 3) conditions.

Morphogens may be used in diagnostic applications by comparing the quantity of morphogen present in a body fluid sample with a predetermined reference value, with fluctuations in fluid morphogen levels indicating a change in the status of nerve tissue. Alternatively, fluctuations in the level of endogenous morphogen antibodies may be detected by this method, most likely in serum, using an antibody or other binding protein capable of interacting specifically with the endogenous morphogen antibody. Detected fluctuations in the levels of the endogenous antibody may be used as indicators of a change in tissue status.

Example 10. <u>Alleviation of Immune Response-Mediated Nerve Tissue Damage</u>

The morphogens described herein may be used to alleviate immunologically-related damage to nerve tissue. Details of this damage and the use of morphogens to alleviate this injury are disclosed in copending USSN 753,059, filed August 30, 1991, the disclosure of which is incorporated herein. A primary source of such damage to nerve tissue follows hypoxia or ischemia-reperfusion of a blood supply to a neural pathway, such as may result from an embolic stroke, or may be induced during a surgical procedure. As described in USSN 753,059, morphogens have been shown to alleviate damage to myocardial tissue following ischemia-reperfusion of the blood supply to the tissue. The effect of morphogens on alleviating immunologically-related damage to nerve tissue may be assessed using methodologies and models known to those skilled in the art and described below.

For example, the rabbit embolic stroke model provides a useful method for assessing the effect of morphogens on tissue injury following cerebral ischemia-reperfusion. The protocol disclosed below is essentially that of Phillips et al. (1989) <u>Annals of Neurology</u> <u>25</u>:281-285, the disclosure of which is herein incorporated by reference. Briefly, white New England rabbits (2-3kg) are anesthetized and placed on a respirator. The intracranial circulation then is selectively catheterized by the Seldinger technique. Baseline cerebral angiography then is performed, employing a digital substration unit. The distal internal carotid artery or its branches then is selectively embolized with 0.035 ml of 18-hour-aged

autologous thrombus. Arterial occlusion is documented by repeat angiography immediately after embolization. After a time sufficient to induce cerebral infarcts (15 minutes or 90 minutes), reperfusion is induced by administering a bolus of a reperfusion agent such as the TPA analogue FB-FB-CF (e.g., 0.8 mg/kg over 2 minutes).

The effect of morphogen on cerebral infarcts can be assessed by administering varying concentrations of morphogens, e.g., OP-1, at different times following embolization and/or reperfusion. The rabbits are sacrificed 3-14 days post embolization and their brains prepared for neuropathological examination by fixing by immersion in 10% neutral buffered formation for at least 2 weeks. The brains then are sectioned in a coronal plane at 2-3 mm intervals, numbered and submitted for standard histological processing in paraffin, and the degree of nerve tissue necrosis determined visually. Morphogen-treated animals are anticipated to reduce or significantly inhibit nerve tissue necrosis following cerebral ischemia-reperfusion in the test animals as determined by histology comparison with nontreated animals.

Example 11. Animal Model for Assessing Morphogen Efficacy *In Vivo*

The *in vivo* activities of the morphogens described herein also are assessed readily in an animal model as described herein. A suitable animal, preferably exhibiting nerve tissue damage, for example, genetically or environmentally induced, is injected intracerebrally with an effective amount of a morphogen in a suitable therapeutic formulation, such as

phosphate-buffered saline, pH 7. The morphogen preferably is injected within the area of the affected neurons. The affected tissue is excised at a subsequent time point and the tissue evaluated morphologically and/or by evaluation of an appropriate biochemical marker (e.g., by morphogen or N-CAM localization; or by measuring the dose-dependent effect on a biochemical marker for CNS neurotrophic activity or for CNS tissue damage, using for example, glial fibrillary acidic protein as the marker. The dosage and incubation time will vary with the animal to be tested. Suitable dosage ranges for different species may be determined by comparison with established animal models. Presented below is an exemplary protocol for a rat brain stab model.

Briefly, male Long Evans rats, obtained from standard commercial sources, are anesthesized and the head area prepared for surgery. The calvariae is exposed using standard surgical procedures and a hole drilled toward the center of each lobe using a 0.035K wire, just piercing the calvariae. 25μl solutions containing either morphogen (e.g., OP-1, 25μg) or PBS then is provided to each of the holes by Hamilton syringe. Solutions are delivered to a depth approximately 3 mm below the surface, into the underlying cortex, corpus callosum and hippocampus. The skin then is sutured and the animal allowed to recover.

Three days post surgery, rats are sacrificed by decapitation and their brains processed for sectioning. Scar tissue formation is evaluated by immunofluoresence staining for glial fibrillary acidic protein, a marker protein for glial scarring, to qualitatively determine

the degree of scar formation. Glial fibrillary acidic protein antibodies are available commercially, e.g., from Sigma Chemical Co., St. Louis, MO. Sections also are probed with anti-OP-1 antibodies to determine the presence of OP-1. Reduced levels of glial fibrillary acidic protein are anticipated in the tissue sections of animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation and stimulate nerve regeneration.

Example 12. In Vitro Model for Evaluating Morphogen Species Transport Across the Blood-Brain Barrier.

Described below is an *in vitro* method for evaluating the facility with which selected morphogen species likely will pass across the blood-brain barrier. A detailed description of the model and protocol are provided by Audus et al. (1987) Ann. N.Y. Acad. Sci. 507:9-18, the disclosure of which is incorporated herein by reference.

Briefly, microvessel endothelial cells are isolated from the cerebral gray matter of fresh bovine brains. Brains are obtained from a local slaughter house and transported to the laboratory in ice cold minimum essential medium (MEM) with antibiotics. Under sterile conditions the large surface blood vessels and meninges are removed using standard dissection procedures. The cortical gray matter is removed by aspiration, then minced into cubes of about 1mm. The minced gray matter then is incubated with 0.5% dispase (BMB, Indianapolis, IN) for 3 hours at 37° C in a shaking water bath. Following the 3 hour digestion, the mixture is concentrated by centrifugation (1000 x g for 10 min.),

then resuspended in 13% dextran and centrifuged for 10 min. at 5800 x g. Supernatant fat, cell debris and myelin are discarded and the crude microvessel pellet resuspended in 1 mg/ml collagenase/dispase and incubated in a shaking water bath for 5 hours at 37° C. After the 5-hour digestion, the microvessel suspension is applied to a pre-established 50% Percoll gradient and centrifuged for 10 min at 1000 x g. The band containing purified endothelial cells (second band from the top of the gradient) is removed and washed two times with culture medium (e.g., 50% MEM/50% F-12 nutrient mix). The cells are frozen (-80° C.) in medium containing 20% DMSO and 10% horse serum for later use.

After isolation, approximately 5 x 10^5 cells/cm^2 are plated on culture dishes or 5-12 mμ pore size polycarbonate filters that are coated with rat collagen and fibronectin. 10-12 days after seeding the cells, cell monolayers are inspected for confluency by microscopy.

Characterization of the morphological, histochemical and biochemical properties of these cells has shown that these cells possess many of the salient features of the blood-brain barrier. These features include: tight intercellular junctions, lack of membrane fenestrations, low levels of pinocytotic activity, and the presence of gamma-glutamyl transpeptidase, alkaline phosphatase, and Factor VIII antigen activities.

The cultured cells can be used in a wide variety of experiments where a model for polarized binding or transport is required. By plating the cells in

multi-well plates, receptor and non-receptor binding of both large and small molecules can be conducted. In order to conduct transendothelial cell flux measurements, the cells are grown on porous polycarbonate membrane filters (e.g., from Nucleopore, Pleasanton, CA). Large pore size filters (5-12 mμ) are used to avoid the possibility of the filter becoming the rate-limiting barrier to molecular flux. The use of these large-pore filters does not permit cell growth under the filter and allows visual inspection of the cell monolayer.

Once the cells reach confluency, they are placed in a side-by-side diffusion cell apparatus (e.g., from Crown Glass, Sommerville, NJ). For flux measurements, the donor chamber of the diffusion cell is pulsed with a test substance, then at various times following the pulse, an aliquot is removed from the receiver chamber for analysis. Radioactive or fluorescently-labelled substances permit reliable quantitation of molecular flux. Monolayer integrity is simultaneously measured by the addition of a non-transportable test substance such as sucrose or inulin and replicates of at least 4 determinations are measured in order to ensure statistical significance.

Example 13. Screening Assay for Candidate Compounds which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to affect the level of a given morphogen may be found using the following screening assay, in which the level of morphogen production by a cell type which produces measurable levels of the morphogen is determined with and without incubating the cell in culture with the

compound, in order to assess the effects of the compound on the cell. This can be accomplished by detection of the morphogen either at the protein or RNA level. A more detailed description also may be found in USSN 752,861, incorporated hereinabove by reference.

13.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described widely in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 well or 24 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or other growth factors).

Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for OP-1 production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis. To monitor <u>de</u>

novo OP-1 synthesis, some cultures are labeled according to conventional procedures with an ^{35}S-methionine/^{35}S-cysteine mixture for 6-24 hours and then evaluated to OP-1 synthesis by conventional immunoprecipitation methods.

13.2 Determination of Level of Morphogenic Protein

In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

1 μg/100 μl of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μl aliquot of an appropriate dilution of each of the test samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 μl biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100 μl strepavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in

adjuvant and is given subcutaneously. The second injection contains 50 μg of OP-1 in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 μg of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, both mice are boosted intraperitoneally with 100 μg of OP-1 (307-431) and 30 μg of the N-terminal peptide (Ser_{293}-Asn_{309}-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

adjuvant and is given subcutaneously. The second injection contains 50 μg of OP-1 in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 μg of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, both mice are boosted intraperitoneally with 100 μg of OP-1 (307-431) and 30 μg of the N-terminal peptide (Ser_{293}-Asn_{309}-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR

(iii) NUMBER OF SEQUENCES: 33

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patent In Release #1.0, Version #1.25

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(B) FILING DATE: 11-MAR-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 752,764
(B) FILING DATE:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: Generic Sequence 1

(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α-amino acids or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
                    Xaa Xaa Xaa Xaa Xaa Xaa
                     1               5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            10                  15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
        20                  25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    30                  35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
40                   45                  50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
                55                  60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            65                  70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        75                  80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
    85                  90
Xaa Cys Xaa
 95
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 2

(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α-amino acids or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
                        Xaa Xaa Xaa Xaa Xaa Xaa
                         1               5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
               10                  15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
          20                  25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
     30                  35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
40                  45                  50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
                    55                  60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
               65                  70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
          75                  80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
     85                  90
Xaa Cys Xaa
 95
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 3

(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
    Leu Tyr Val Xaa Phe
    1               5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                10
Xaa Ala Pro Gly Xaa Xaa Xaa Ala
 15                 20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        25                  30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
        40                  45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
     55                 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
            65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 70                 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
            80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 85                 90
```

Xaa Cys Gly Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: Generic Sequence 4
(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
 1               5                  10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                15
Xaa Ala Pro Xaa Gly Xaa Xaa Ala
 20                 25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        30                  35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                40
Asn Xaa Xaa Asn His Ala Xaa Xaa
        45                  50
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                55
```

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
60 65

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
70

Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85

Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95

Xaa Cys Gly Cys Xaa
100

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: hOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Thr Gly Ser Lys Gln Arg Ser Gln
1 5

Asn Arg Ser Lys Thr Pro Lys Asn Gln
10 15

Glu Ala Leu Arg Met Ala Asn Val Ala
20 25

Glu Asn Ser Ser Ser Asp Gln Arg Gln
30 35

Ala Cys Lys Lys His Glu Leu Tyr Val
40 45

Ser Phe Arg Asp Leu Gly Trp Gln Asp
50

Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	65					70		
Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
			85					90
Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
				95				
Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
100					105			
Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	110					115		
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
		120					125	
Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala
			130					135
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: mOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser	Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln
1				5				

```
Asn  Arg  Ser  Lys  Thr  Pro  Lys  Asn  Gln
 10                       15
Glu  Ala  Leu  Arg  Met  Ala  Ser  Val  Ala
      20                       25
Glu  Asn  Ser  Ser  Ser  Asp  Gln  Arg  Gln
           30                       35
Ala  Cys  Lys  Lys  His  Glu  Leu  Tyr  Val
                40                       45
Ser  Phe  Arg  Asp  Leu  Gly  Trp  Gln  Asp
                     50
Trp  Ile  Ile  Ala  Pro  Glu  Gly  Tyr  Ala
 55                       60
Ala  Tyr  Tyr  Cys  Glu  Gly  Glu  Cys  Ala
      65                       70
Phe  Pro  Leu  Asn  Ser  Tyr  Met  Asn  Ala
           75                       80
Thr  Asn  His  Ala  Ile  Val  Gln  Thr  Leu
                85                       90
Val  His  Phe  Ile  Asn  Pro  Asp  Thr  Val
                     95
Pro  Lys  Pro  Cys  Cys  Ala  Pro  Thr  Gln
100                      105
Leu  Asn  Ala  Ile  Ser  Val  Leu  Tyr  Phe
     110                      115
Asp  Asp  Ser  Ser  Asn  Val  Ile  Leu  Lys
          120                      125
Lys  Tyr  Arg  Asn  Met  Val  Val  Arg  Ala
               130                      135
Cys  Gly  Cys  His
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: hOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala	Val	Arg	Pro	Leu	Arg	Arg	Arg	Gln
1				5				
Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln
10					15			
Ala	Asn	Arg	Leu	Pro	Gly	Ile	Phe	Asp
	20					25		
Asp	Val	His	Gly	Ser	His	Gly	Arg	Gln
		30					35	
Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val
			40					45
Ser	Phe	Gln	Asp	Leu	Gly	Trp	Leu	Asp
				50				
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ser
	65					70		
Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu
			85					90
Val	His	Leu	Met	Lys	Pro	Asn	Ala	Val
				95				
Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
100					105			
Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr
	110					115		
Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg
		120					125	
Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala
			130					135
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: mOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala	Ala	Arg	Pro	Leu	Lys	Arg	Arg	Gln
1				5				
Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His
10					15			
Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp
	20					25		
Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	Glu
		30					35	
Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val
			40					45
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp
				50				
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	65					70		
Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu
			85					90
Val	His	Leu	Met	Lys	Pro	Asp	Val	Val
				95				
Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
100					105			

Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr
	110					115		
Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg
		120					125	
Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala
			130					135
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: CBMP-2A(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	Asp	Phe	Ser
1				5					10	
Asp	Val	Gly	Trp	Asn	Asp	Trp	Ile	Val	Ala	Pro
			15					20		
Pro	Gly	Tyr	His	Ala	Phe	Tyr	Cys	His	Gly	Glu
		25					30			
Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser
	35					40				
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	Asn
45					50					55
Ser	Val	Asn	Ser	Lys	Ile	Pro	Lys	Ala	Cys	Cys
				60					65	

Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
70 75
Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys
80 85
Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly
90 95
Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 101 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: CBMP-2B(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Arg Arg His Ser
1 5
Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
10 15
Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala
20 25
Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
30 35
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
40 45
Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser
50 55 60
Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu
65 70

Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Tyr
75 80
Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
85 90
Val Val Glu Gly Cys Gly Cys Arg
95 100

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: DPP(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser
1 5 10
Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro
15 20
Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly Lys
25 30
Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser
35 40
Thr Asn His Ala Val Val Gln Thr Leu Val Asn
45 50 55
Asn Asn Asn Pro Gly Lys Val Pro Lys Ala Cys
60 65
Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met
70 75

Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu
        80   85
Lys Asn Tyr Gln Glu Met Thr Val Val Gly Cys
    90   95
Gly Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Vgl(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys
 1   5   10
Asp Val Gly Trp Gln Asn Trp Val Ile Ala Pro
            15   20
Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly Glu
        25   30
Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly
    35   40
Ser Asn His Ala Ile Leu Gln Thr Leu Val His
45   50   55
Ser Ile Glu Pro Glu Asp Ile Pro Leu Pro Cys
                60   65
Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met
            70   75

Leu Phe Tyr Asp Asn Asn Asp Asn Val Val Leu
        80   85
Arg His Tyr Glu Asn Met Ala Val Asp Glu Cys
    90   95
Gly Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Vgr-1(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln
  1   5   10
Asp Val Gly Trp Gln Asp Trp Ile Ile Ala Pro
            15   20
Xaa Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu
        25   30
Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala
    35   40
Thr Asn His Ala Ile Val Gln Thr Leu Val His
 45   50   55
Val Met Asn Pro Glu Tyr Val Pro Lys Pro Cys
                60   65
Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val
            70   75

Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu
80 85

Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys
90 95

Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: protein
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(F) TISSUE TYPE: BRAIN

(ix) FEATURE:
(D) OTHER INFORMATION:
/product= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
1 5 10

Trp His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr
15 20 25

Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
30 35 40

Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
45 50 55

Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
60 65 70

Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
75 80 85

Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
90 95 100

Cys Arg
105

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Cys Xaa Xaa Xaa Xaa
 1               5
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..1341
(D) OTHER INFORMATION:/standard_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG      57
                                                     Met His Val
                                                      1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA     105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
    5                   10                  15

CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC     153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
 20                 25                  30                  35

GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG     201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
                40                  45                  50
```

```
CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC      249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
             55                  60                  65

CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG      297
Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
         70                  75                  80

CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC      345
Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
     85                  90                  95

GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC      393
Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
100                 105                 110                 115

CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC      441
Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp
                120                 125                 130

ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC      489
Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe
            135                 140                 145

CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC      537
His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile
        150                 155                 160

CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC      585
Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp
    165                 170                 175

TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT      633
Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr
180                 185                 190                 195

CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC      681
Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu
                200                 205                 210

GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC      729
Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp
            215                 220                 225

ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG      777
Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu
        230                 235                 240

GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC      825
Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro
    245                 250                 255
```

AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC 873
Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro
260 265 270 275

TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC 921
Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile
280 285 290

CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC 969
Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro
295 300 305

AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC 1017
Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser
310 315 320

AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC 1065
Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe
325 330 335

CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC 1113
Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala
340 345 350 355

GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG 1161
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met
360 365 370

AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC 1209
Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn
375 380 385

CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC 1257
Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
390 395 400

ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA 1305
Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
405 410 415

TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC 1351
Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG 1411

GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG 1471

TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC 1531

ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591

GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651

CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711

GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771

CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A 1822

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 431 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /Product="OP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1               5                  10                  15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
            20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
        35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
    50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65                  70                  75                  80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
                85                  90                  95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
            100                 105                 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
        115                 120                 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
    130                 135                 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145                 150                 155                 160
```

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175

Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
195 200 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
275 280 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
290 295 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305 310 315 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
325 330 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
340 345 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1873 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 104..1393
(D) OTHER INFORMATION: /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG 60

CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC 115
Met His Val Arg
1

TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT 163
Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro
5 10 15 20

CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG 211
Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
25 30 35

GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG 259
Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg
40 45 50

GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG 307
Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro
55 60 65

CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG 355
Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu
70 75 80

GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG 403
Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln
85 90 95 100

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GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT      451
Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro
                105                 110                 115

TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC      499
Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val
            120                 125                 130

ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT      547
Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro
        135                 140                 145
CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG      595
Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu
    150                 155                 160

GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC      643
Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile
165                 170                 175                 180

CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG      691
Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val
                185                 190                 195

CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC      739
Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser
            200                 205                 210

CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA      787
Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr
        215                 220                 225

GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA      835
Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu
    230                 235                 240

CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG      883
Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu
245                 250                 255                 260

GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG      931
Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met
                265                 270                 275

GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC      979
Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser
            280                 285                 290

ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC     1027
Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn
        295                 300                 305
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CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC      1075
Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp
    310                 315                 320

CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC      1123
Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp
325                 330                 335                 340

CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC      1171
Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr
                345                 350                 355

TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC      1219
Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala
            360                 365                 370

ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC      1267
Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp
        375                 380                 385

ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT      1315
Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser
    390                 395                 400

GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA      1363
Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg
405                 410                 415                 420

AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG         1413
Asn Met Val Val Arg Ala Cys Gly Cys His
                425                 430

ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG    1473

CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG    1533

AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT    1593

GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT    1653

GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTTGAGGAGT    1713

AATCGCAAGC CTCGTTCAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG    1773

TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT    1833

GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC                          1873
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 430 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /product= "mOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1               5                  10                  15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
            20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
        35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
    50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65                  70                  75                  80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
                85                  90                  95

Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
            100                 105                 110

Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
        115                 120                 125

Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
    130                 135                 140

Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
145                 150                 155                 160

Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
                165                 170                 175

Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
            180                 185                 190

Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
        195                 200                 205
```

Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
210 215 220

Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
225 230 235 240

Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
245 250 255

Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
260 265 270

Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
275 280 285

Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
290 295 300

Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
305 310 315 320

Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
325 330 335

Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
340 345 350

Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
355 360 365

Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
370 375 380

Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
385 390 395 400

Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
405 410 415

Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi)ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: HIPPOCAMPUS

(ix)FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 490..1696
(D) OTHER INFORMATION: /note= "hOP2 (cDNA)"

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA     60

GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCCAGG AGGCGCTGGA GCAACAGCTC    120

CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC    180

GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT    240

CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG    300

GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC    360

CGCCCCGCCC CGCCGCCCGC CGCCCGCCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC    420

AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC    480

CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG         528
          Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
            1               5                  10

GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC      576
Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro
     15                  20                  25

GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG      624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln
 30                  35                  40                  45

CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC      672
Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg
                 50                  55                  60

GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG      720
Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met
             65                  70                  75

CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG      768
Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala
         80                  85                  90
```

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CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT      816
Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val
     95                 100                 105

AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG      864
Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp
110                 115                 120                 125

AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC      912
Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val
                130                 135                 140

ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC      960
Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu
            145                 150                 155

AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC     1008
Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser
        160                 165                 170

AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT     1056
Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala
    175                 180                 185

GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC     1104
Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys
190                 195                 200                 205

TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG     1152
Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu
                210                 215                 220

ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT     1200
Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly
            225                 230                 235

CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG     1248
Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg
        240                 245                 250

GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG     1296
Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg
    255                 260                 265

AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC     1344
Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu
270                 275                 280                 285

CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC     1392
Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys
                290                 295                 300
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```
CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC     1440
Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp
            305                 310                 315

TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG     1488
Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu
        320                 325                 330

TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC     1536
Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile
    335                 340                 345

CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG     1584
Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala
350                 355                 360                 365

TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC     1632
Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp
                370                 375                 380

AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG     1680
Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys
            385                 390                 395

GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG                 1723
Ala Cys Gly Cys His
        400
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 402 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) OTHER INFORMATION: /product= "hOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1               5                  10                  15

Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
            20                  25                  30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
        35                  40                  45
```

```
Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
     50                  55                  60

Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
 65                  70                  75                  80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
                 85                  90                  95

Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
            100                 105                 110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
        115                 120                 125

Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
    130                 135                 140

Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
145                 150                 155                 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
                165                 170                 175

Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
            180                 185                 190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
        195                 200                 205

Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
    210                 215                 220

Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
225                 230                 235                 240

Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
                245                 250                 255

Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
            260                 265                 270

Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
        275                 280                 285

Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
    290                 295                 300

Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
305                 310                 315                 320
```

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Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
                325                 330                     335

Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
            340                 345                     350

Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
        355                 360                     365

Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
    370                 375                     380

Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly
385                 390                     395                     400

Cys His
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1926 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 93..1289
(D) OTHER INFORMATION: /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
        GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC          50

CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT     104
                                               Met Ala Met Arg
                                                 1

CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC    152
Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly
  5                 10                  15                  20

GGC CAC GGT CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA    200
Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly
                25                  30                  35
```

GCG CGC GAG CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG 248
Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly
40 45 50

CTA CCG GGA CGG CCC CGA CCC CGT GCA CAA CCC GCG GCT GCC CGG CAG 296
Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln
55 60 65

CCA GCG TCC GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC 344
Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr
70 75 80

GAT GAC GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC 392
Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp
85 90 95 100

CTG GTC ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC 440
Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly
105 110 115

TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC 488
Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile
120 125 130

CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA 536
Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu
135 140 145

CCC AGC ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA 584
Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu
150 155 160

GTG GTC CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT 632
Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp
165 170 175 180

CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC 680
Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile
185 190 195

ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA 728
Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly
200 205 210

CTC CGC CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC 776
Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly
215 220 225

CTG GCT GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC 824
Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe
230 235 240

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ATG GTA ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG        872
Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg
245                 250                 255                 260

GCA GCG AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT        920
Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
                265                 270                 275

CCG CAC CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC        968
Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
            280                 285                 290

CGC GGC AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT       1016
Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
        295                 300                 305

GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC       1064
Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
    310                 315                 320

TAT TAC TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC       1112
Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
325                 330                 335                 340

GCC ACC AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA       1160
Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
                345                 350                 355

GAT GTT GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC       1208
Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
            360                 365                 370

TCT GTG CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC       1256
Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
        375                 380                 385

CGT AAC ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC     1309
Arg Asn Met Val Val Lys Ala Cys Gly Cys His
    390                 395

TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT     1369

TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCTGCTA     1429

AAATTCTGGT CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC     1489

CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA     1549

ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC     1609

CTCAGCCCAC AATGGCAAAT TCTGGATGGT CTAAGAAGGC CGTGGAATTC TAAACTAGAT     1669
```

GATCTGGGCT CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTTAGGT ATAACAGACA 1729

CATACACTTA GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA 1789

AGAATCAGAG CCAGGTATAG CGGTGCATGT CATTAATCCC AGCGCTAAAG AGACAGAGAC 1849

AGGAGAATCT CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA 1909

AAAAAAAAAC GGAATTC 1926

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
  1               5                  10                  15

Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
             20                  25                  30

Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala
         35                  40                  45

Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala
 50                  55                  60                  65

Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala
                 70                  75                  80

Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg
         85                  90                  95

Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr
    100                 105                 110

Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr
115                 120                 125                 130

Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
                135                 140                 145
```

Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met
150 155 160

Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe
165 170 175

Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu
180 185 190

Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp
195 200 205 210

Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp
215 220 225

Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln
230 235 240

Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala
245 250 255

Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn
260 265 270

Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His
275 280 285 290

Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser
295 300 305

Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr
310 315 320

Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys
325 330 335

Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met
340 345 350

Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser
355 360 365 370

Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
375 380 385

Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
390 395

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1368 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1368
(D) OTHER INFORMATION:/STANDARD NAME="60A"

(x) PUBLICATION INFORMATION:
(A) AUTHORS: WHARTON, KRISTI A.; THOMSEN, GERALD H.; GELBERT, WILLIAM M.
(B) TITLE: DROSOPHILA 60A GENE...
(C) JOURNAL: PROC. NAT'L ACAD. SCI. USA
(D) VOLUME: 88
(E) RELEVANT RESIDUES IN SEQ ID NO:3: FROM 1 TO 1368
(F) PAGES: 9214-9218
(G) DATE: OCT - 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC      48
Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
  1               5                  10                  15

CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG      96
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
             20                  25                  30

GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC     144
Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
         35                  40                  45

CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC     192
Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
     50                  55                  60

TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC     240
Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
 65                  70                  75                  80

CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG     288
Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
                 85                  90                  95

CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG     336
Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
            100                 105                 110
```

```
GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC      384
Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
        115                 120                 125

GAC CTC GAG GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC      432
Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
    130                 135                 140

CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG      480
Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145                 150                 155                 160

AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT      528
Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
                165                 170                 175

CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG      576
Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
            180                 185                 190

ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG      624
Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
        195                 200                 205

ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC      672
Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
    210                 215                 220

ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC      720
Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
225                 230                 235                 240

GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC      768
Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
                245                 250                 255

GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA      816
Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
            260                 265                 270

CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA      864
His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
        275                 280                 285

CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC      912
Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
    290                 295                 300

TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GCC CAC AGC AGC CAC      960
Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
305                 310                 315                 320
```

```
CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG     1008
His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
                325                 330                 335

GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC     1056
Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
            340                 345                 350

AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG     1104
Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
        355                 360                 365

CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC     1152
His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
    370                 375                 380

GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT     1200
Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
385                 390                 395                 400

GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC     1248
Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
                405                 410                 415

AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC     1296
Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
            420                 425                 430

CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT     1344
His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
        435                 440                 445

GTG AAA TCC TGC GGG TGC CAT TGA                                     1368
Val Lys Ser Cys Gly Cys His
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 455 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
 1               5                  10                  15
```

Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
20 25 30

Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
35 40 45

Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
50 55 60

Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
65 70 75 80

Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
85 90 95

Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
100 105 110

Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
115 120 125

Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
130 135 140

Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145 150 155 160

Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
165 170 175

Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
180 185 190

Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
195 200 205

Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
210 215 220

Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
225 230 235 240

Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
245 250 255

Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
260 265 270

His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
275 280 285

Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
290 295 300

```
Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
305                 310                 315                 320

His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
                325                 330                 335

Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
            340                 345                 350

Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
        355                 360                 365

His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
    370                 375                 380

Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
385                 390                 395                 400

Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
                405                 410                 415

Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
            420                 425                 430

His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
        435                 440                 445

Val Lys Ser Cys Gly Cys His
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:
(A) ORGANISM: Homo Sapiens

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note="BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

(i)SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..104
(D) OTHER INFORMATION: /note="BMP3"

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
1               5                   10                  15

Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Try Cys Ser Gly
            20                  25                  30

Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
        35                  40                  45

Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
    50                  55                  60

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65                  70                  75                  80

Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
                85                  90                  95

Thr Val Glu Ser Cys Ala Cys Arg
            100
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1 5 10 15

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
85 90 95

Arg Ser Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note= "BMP6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1 5 10 15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

```
Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
    50                  55                  60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65                  70                  75                  80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
                85                  90                  95

Arg Ala Cys Gly Cys His
            100
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN XAA AT EACH POS'N IS INDEPENDENTLY SELECTED FROM THE RESIDUES OCCURRING AT THE CORRESPONDING POS'N IN THE C-TERMINAL SEQUENCE OF MOUSE OR HUMAN OP1 OR OP2 (SEE SEQ. ID NOS. 5,6,7 and 8 or 16,18,20 and 22.)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1               5                   10                  15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
            20                  25                  30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
        35                  40                  45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
    50                  55                  60
```

Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85 90 95

Xaa Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:30:

(i)SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii)MOLECULE TYPE: protein
(ix)FEATURE:
(A) NAME: Generic Sequence 5
(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:30:

Leu Xaa Xaa Xaa Phe
1 5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
10
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
15 20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Xaa Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 102 amino acids
    (B) TYPE: amino acids
    (C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
    (A) NAME: Generic Sequence 6
    (D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
 1               5                   10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                15
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
 20                 25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        30                  35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
        45                  50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
    60                  65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
            70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 75                 80
```

```
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
            85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 90                 95
Xaa Cys Xaa Cys Xaa
        100
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1238 base pairs, 372 amino acids
(B) TYPE: nucleic acid, amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) ORIGINAL SOURCE:
(A) ORGANISM: human
(F) TISSUE TYPE: BRAIN

(iv) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:
(D) OTHER INFORMATION:
/product= "GDF-1"
/note= "GDF-1 CDNA"

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Lee, Se-Jin
(B) TITTLE: Expression of Growth/Differentiation Factor 1
(C) JOURNAL: Proc. Nat'l Acad. Sci.
(D) VOLUME: 88
(E) RELEVANT RESIDUES: 1-1238
(F) PAGES: 4250-4254
(G) DATE: May-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
GGGGACACCG GCCCCGCCCT CAGCCCACTG GTCCCGGGCC GCCGCGGACC CTGCGCACTC      60

TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC GGC     113
                          Met Pro Pro Pro Gln Gln Gly Pro Cys Gly
                          1               5                   10

CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC           158
His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
                15                  20                  25
```

CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC 203
Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu
30 35 40

CAG GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC 248
Gln Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu
45 50 55

CGG CCG GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC 293
Arg Pro Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp
60 65 70

CCC CAG GAG ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC 338
Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val
75 80 85

ACC CTG CAA CCG TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC 383
Thr Leu Gln Pro Cyc His Val Glu Glu Leu Gly Val Ala Gly Asn
90 95 100

ATC GTG CGC CAC ATC CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG 428
Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser
105 110 115

GAG CCT GTC TCG GCC GCG GGG CAT TGC CCT GAG TGG ACA GTC GTC 473
Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr Val Val
120 125 130

TTC GAC CTG TCG GCT GTG GAA CCC GCT GAG CGC CCG AGC CGG GCC 518
Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg Ala
135 140 145

CGC CTG GAG CTG CGT TTC GCG GCG GCG GCG GCG GCA GCC CCG GAG 563
Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Ala Pro Glu
150 155 160

GGC GGC TGG GAG CTG AGC GTG GCG CAA GCG GGC CAG GGC GCG GGC 608
Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly
165 170 175

GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG TTG GTG CCC GCC CTG 653
Ala Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu
180 185 190

GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC GCT TGG GCT CGC 698
Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg
195 200 205

AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG GCG CTA CGC 743
Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg
210 215 220

CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC TCG CTG 788
Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu
225 230 235

CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC CGG 833
Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg
240 245 250

CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC 878
Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly
255 260 265

GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC CAG GTG GGC 923
Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
270 275 280

TGG CAC CGC TGG GTC ATC GCG CCG CGC CCC TTC CTG GCC AAC TAC 968
Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr
285 290 295

TGC CAG GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG 1013
Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
300 305 310

GGG CCG CCG GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC 1058
Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
315 320 325

GCG GCC GCC CCG GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG 1103
Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
330 335 340

CGC CTG TCG CCC ATC TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC 1148
Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
345 350 355

GTG GTG CTG CGG CAG TAT GAG GAC ATG GTG GTG GAC GAG TGC GGC 1193
Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
360 365 370

TGC CGC TAACCCGGGG CGGGCAGGGA CCCGGGCCCA ACAATAAATG CCGCGTGG 1238
Cys Arg
372

(34) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(F) TISSUE TYPE: BRAIN

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:
(D) OTHER INFORMATION: /function=
/product= "GDF-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

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                    1               5                   10

His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
                15                  20                  25

Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu
                30                  35                  40

Gln Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu
                45                  50                  55

Arg Pro Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp
                60                  65                  70

Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val
                75                  80                  85

Thr Leu Gln Pro Cyc His Val Glu Glu Leu Gly Val Ala Gly Asn
                90                  95                  100

Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser
                105                 110                 115

Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr Val Val
                120                 125                 130

Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg Ala
                135                 140                 145

Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Ala Pro Glu
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Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly
165 170 175

Ala Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu
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Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg
195 200 205

Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg
210 215 220

Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu
225 230 235

Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg
240 245 250

Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly
255 260 265

Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
270 275 280

Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr
285 290 295

Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
300 305 310

Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
315 320 325

Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
330 335 340

Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
345 350 355

Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
360 365 370

Cys Arg
372

What is claimed is:

1. A method for enhancing survival of neural cells at risk of dying, the method comprising providing a morphogen to said cells at a concentration and for a time sufficient to enhance survival of said cells.

2. The method of claim 1 wherein said cells are at risk of dying due to chemical or mechanical trauma to nerve tissue comprising said cells.

3. The method of claim 2 wherein said trauma comprises a transected nerve.

4. The method of claim 2 wherein said morphogen is provided to said cells prior to said trauma.

5. The method of claim 2 wherein said trauma results in demyelination of said cells.

6. The method of claim 2 wherein said trauma results from exposure of said cells to a cellular toxin.

7. The method of claim 6 wherein said toxin comprises ethanol.

8. The method of claim 1 wherein said cells are at risk of dying due to a neuropathy.

9. The method of claim 8 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.

10. The method of claim 9 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis or Alzheimer's disease.

11. The method of claim 1 wherein said cells are at risk of dying due a neoplastic lesion associated with nerve tissue comprising said cells.

12. The method of claim 11 wherein said lesion results from a neoplasm comprising cells of neuronal origin.

13. The method of claim 12 wherein said neoplasm comprises a neuroblastoma or a retinoblastoma.

14. The method of claim 11 wherein said lesion results from a neoplasm comprising glial cells.

15. The method of claim 1 wherein said neural cells at risk of dying comprise part of the central nervous system.

16. The method of claim 15 wherein said cells comprise striatal basal ganglia neurons.

17. The method of claim 15 wherein said cells comprise neurons of the substantia nigra.

18. The method of claim 1 wherein said cells at risk of dying comprise part of the peripheral nervous system.

19. The method of claim 1 wherein said morphogen stimulates cell adhesion molecule production in said cells.

20. The method of claim 19 wherein said cell adhesion molecule is a nerve cell adhesion molecule.

21. The method of claim 20 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.

22. The method of claim 1 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

23. The method of claim 22 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).

24. The method of claim 23 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

25. The method of claim 24 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

26. The method of claim 21 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

27. A method for enhancing the survival of neural cells at risk of dying in a mammal, the method comprising the step of administering to said mammal an effective amount of an agent capable of stimulating production of an endogenous morphogen.

28. The method of claim 27 wherein said agent stimulates production of an endogenous morphogen in the tissue comprising said neural cells.

29. A method for maintaining a neural pathway in a mammal, comprising:

providing a morphogen to the neurons defining said pathway at a concentration and for a time sufficient to maintain said pathway.

30. The method of claim 29 wherein said morphogen is provided prior to injury to said pathway.

31. The method of claim 29 wherein said morphogen is sufficient to stimulate repair of a damaged neural pathway.

32. The method of claim 31 wherein said damaged neural pathway results from mechanical or chemical trauma to said pathway.

33. The method of claim 32 wherein said trauma comprises a severed nerve.

34. The method of claim 32 wherein said trauma comprises demyelination of the neurons defining said pathway.

35. The method of claim 32 wherein said trauma results from exposure of the cells defining said pathway to a cellular toxin.

36. The method of claim 35 wherein said toxin comprises ethanol.

37. The method of claim 29 wherein said damaged neural pathway results from a neuropathy of the cells defining said pathway.

38. The method of claim 37 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.

39. The method of claim 38 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, or Alzheimer's disease.

40. The method of claim 37 wherein said neuropathy comprises axonal degeneration.

41. The method of claim 37 wherein said neuropathy comprises a demyelinating neuropathy.

42. The method of claim 29 wherein said damaged neural pathway results from a neoplastic lesion.

43. The method of claim 42 wherein said neoplastic lesion is caused by a neuroblastoma or a glioma.

44. The method of claim 29 wherein said morphogen stimulates cell adhesion molecule production in a cell defining said pathway.

45. The method of claim 44 wherein said cell adhesion molecule is a nerve cell adhesion molecule.

46. The method of claim 45 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.

47. The method of claim 29 or 46 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

48. The method of claim 47 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).

49. The method of claim 48 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

50. The method of claim 49 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

51. The method of claim 50 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

52. A method of maintaining a neural pathway in a mammal comprising:

administering said mammal an effective amount of an agent capable of stimulating production of an endogenons morphogen in a cell defining said pathway.

53. A composition for promoting regeneration of a neural pathway at a site of injury in a mammal, comprising:

a biocompatible, in vivo bioresorbable carrier suitable for maintaining a protein at a site in vivo, and

a morphogen, such that said morphogen, when dispersed in said carrier and provided to said site of injury, is capable of stimulating neural pathway regeneration at said site.

54. The composition of claim 53 wherein said carrier is structurally sufficient to assist direction of axonal growth.

55. The composition of claim 54 wherein said carrier comprises a polymeric material.

56. The composition of claim 54 wherein said carrier comprises laminin or collagen.

57. A device for repairing a break in a neural pathway, the device comprising:

a biocompatible tubular casing comprising an exterior and an interior surface and defining a channel through which a neural process may regenerate,

said device having a shape and dimension sufficient to span a break in a neural pathway, and having openings adapted to receive the ends of a severed nerve, and

a morphogen disposed within the channel defined by said tubular casing and accessible to severed nerve ends defining a break in a neural pathway, such that said morphogen stimulates neural pathway regeneration when disposed in said channel and accessible to said nerve ends.

58. The device of claim 57 wherein said morphogen is disposed in said channel together with a biocompatible, bioresorbable carrier suitable for maintaining a protein at a site *in vivo*.

59. The device of claim 58 wherein said carrier comprises sufficient structure to assist direction of axonal growth within said channel.

60. The device of claim 57 wherein the outer surface of said casing is substantially impermeable.

61. The device of claim 58 wherein said carrier comprises a polymer.

62. The device of claim 58 wherein said carrier comprises laminin or collagen.

63. A method for inducing the redifferentiation of transformed cells of neural origin, the method comprising the step of:

contacting said transformed cells with a morphogen composition at a concentration and for a time sufficient to induce redifferentiation of said cells to a morphology characteristic of untransformed neuronal cells.

64. The method of claim 63 wherein said morphology characteristic of untransformed nerve cells includes formation of neurite outgrowths.

65. The method of claim 63 wherein said morphology characteristic of untransformed nerve cells includes cell aggregation and cell adhesion.

66. The method of claim 63 wherein said morphogen composition induces nerve cell adhesion molecule production in said cells.

67. The method of claim 63 wherein said induced nerve cell adhesion molecules include N-CAM-180, N-CAM-140 and N-CAM-120.

68. The method of claim 63 wherein said transformed cells comprise neuroblastoma cells.

69. A method for detecting a neuropathy in a mammal, the method comprising the step of:

detecting fluctuations in the physiological concentration of a morphogen present in the serum or cerebrospinal fluid of said mammal, said fluctuations being indicative of an increase in neuronal cell death.

70. A method for detecting a neuropathy in a mammal, the method comprising the step of:

    detecting fluctuations in the physiological concentration of a morphogen antibody titer present in the serum or cerebraospinal fluid of said mammal, said fluctuations being indicative of an increase in neuronal cell death.

71. The method of claim 69 or 70 wherein said neuropathy results from a neurodegenerative disease, nerve demyelineation, myelin dysfunction, neuronal neoplasias, or nerve trauma.

72. A method of stimulating production of cell adhesion molecules in a tissue comprising the step of:

    providing a morphogen to said tissue for a time and at a concentration sufficient to induce production of cell adhesion molecules in cells of said tissue.

73. The method of claim 72 wherein said cell adhesion molecules comprises nerve cell adhesion molecules.

74. The method of claim 73 wherein said cells comprise neurons.

75. The method of claim 69, 70 or 72 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

76. The method of claim 75 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A (fx).

77. The method of claim 76 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

78. The method of claim 77 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

79. The method of claim 78 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

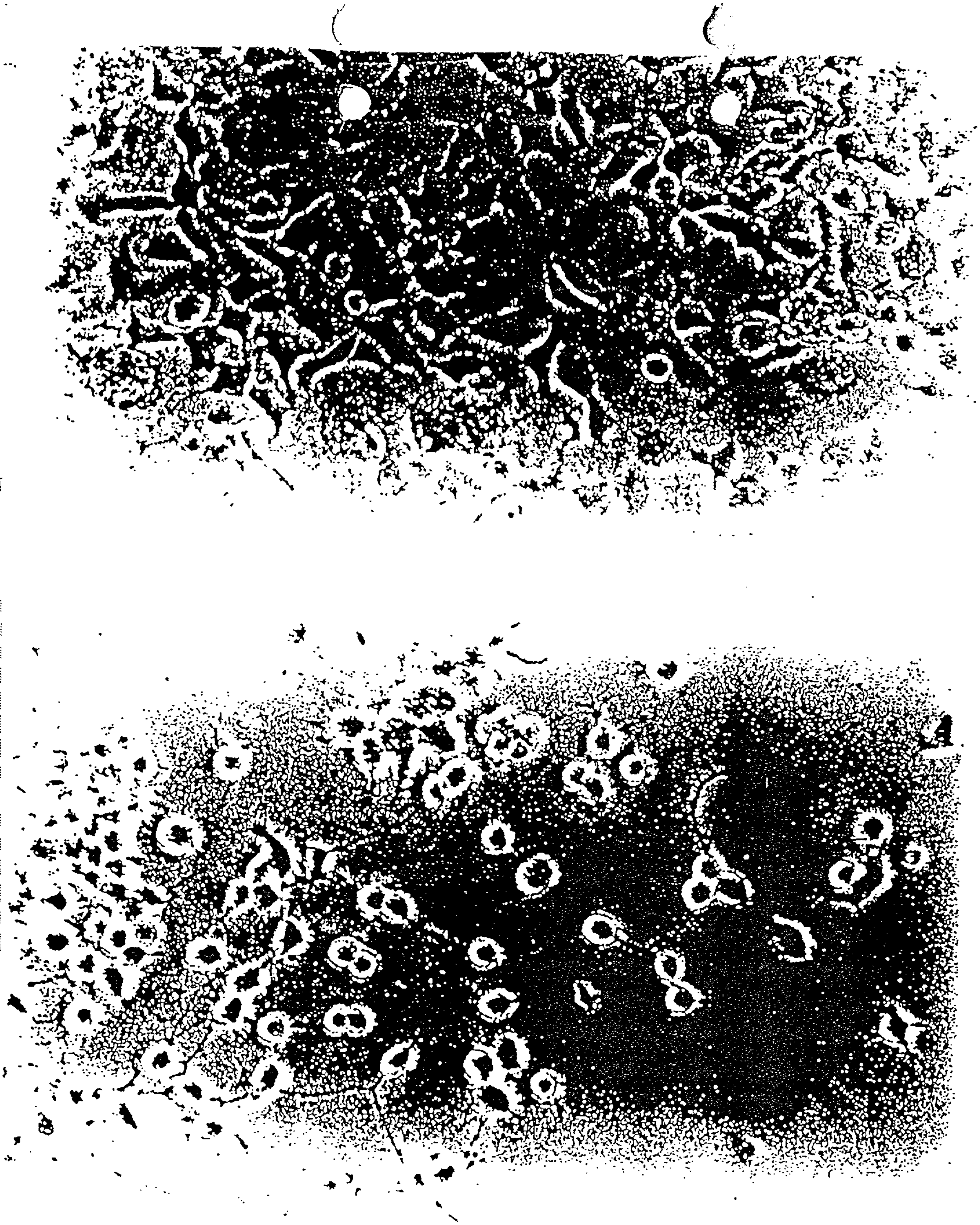
80. A composition for enhancing survival of neuronal cells at risk of dying comprising a morphogen in association with a molecule capable of enhancing the transport of said morphogen across the blood-brain barrier.

81. The invention of claim 53 or 57 wherein said carrier comprises brain tissue derived extracellular matrix.

Abstract of the Disclosure

Disclosed are therapeutic treatment methods, compositions and devices for maintaining neural pathways in a mammal, including enhancing survival of neurons at risk of dying, inducing cellular repair of damaged neurons and neural pathways, and stimulating neurons to maintain their differentiated phenotype. In one embodiment, the invention provides means for stimulating CAM expression in neurons. The invention also provides means for evaluating the status of nerve tissue, including means for detecting and monitoring neuropathies in a mammal. The methods, devices and compositions include a morphogen or morphogen-stimulating agent provided to the mammal in a therapeutically effective concentration.

A



B.

FIG. 1 A-B

OP-1 Differentially Induces 180 and 140 kDa N-CAM

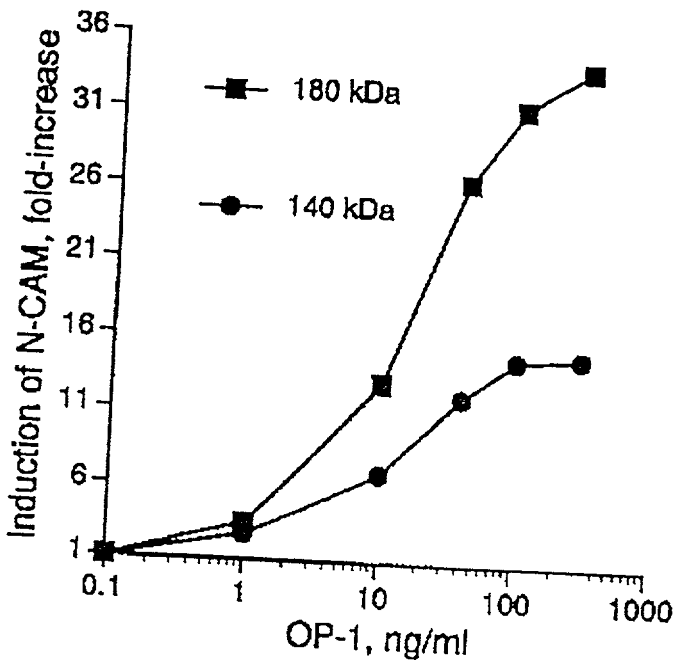


FIG. 2A

Perkdes. Figure 2D

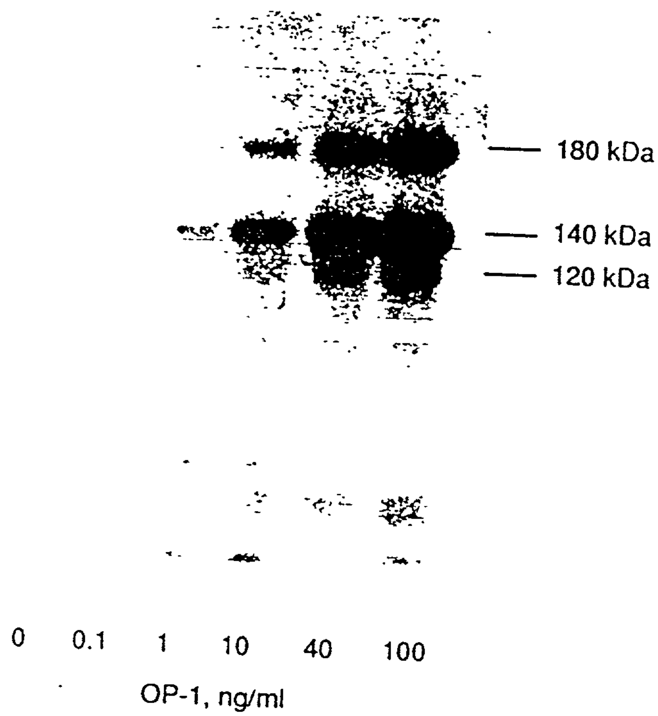
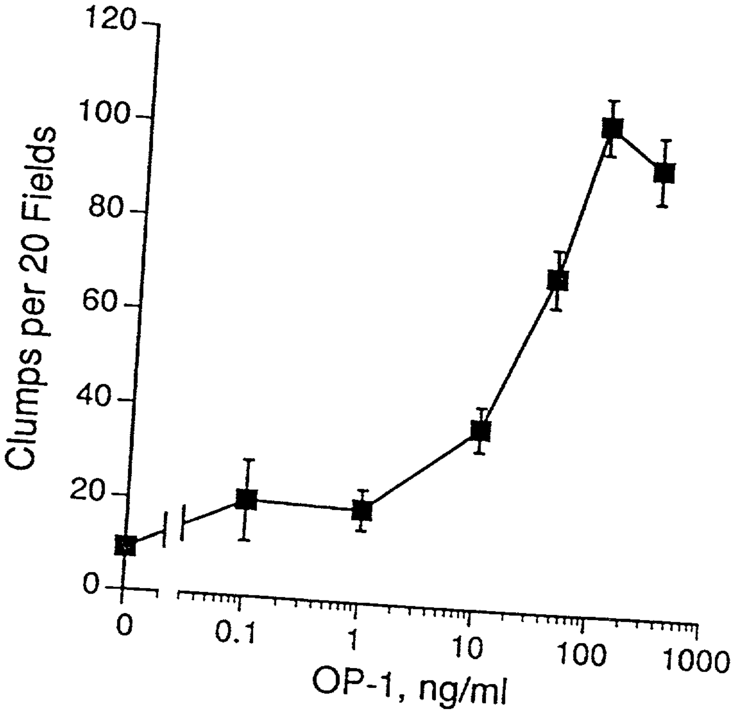


Fig 2B

Perides, Figure 2C



120
100
80
60
40
20
0
Clumps per 20 Fields
0
0.1
1
10
100
1000
OP-1, ng/ml

Fig. 3

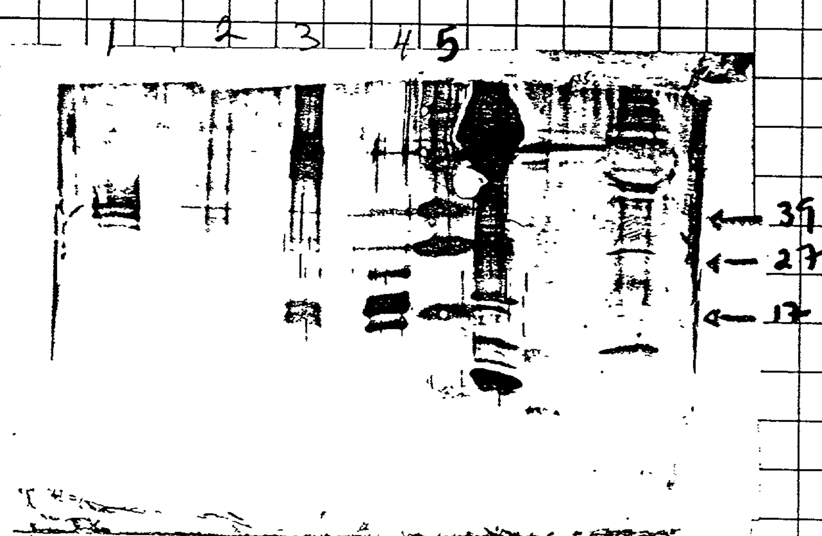


Figure 4